ACE and Subarachnoid Hemorrhage: Strategies for Genetics of Stroke

To the Editor:

I thank Slowik et al for their article investigating the angiotensin-converting enzyme (ACE) insertion/deletion (I/D) polymorphism in subarachnoid hemorrhage (SAH).1 I wish to comment on several aspects of this article related to the design of genetic association studies for complex disorders such as SAH, which are important in interpreting their findings. It is impressive to note the high odds ratios achieved, however this study was less than half the size of the one it aimed to confirm.2 The populations of the 2 studies were of European origin and the allele frequencies were also similar. Using calculations previously described3 and the data from Kermani et al, an estimated sample size of 600 subjects is required to show the presence of an association of ACE I/D with SAH with 80% power. It is surprising that Slowik et al achieved twice as high an odds ratio with half the sample size. The small sample size of this study could have resulted in a type 1 error.

ACE I/D polymorphism has been extensively studied in several disorders with conflicting results. Interestingly, Zhu et al showed that the I/D polymorphism is not functional and its association with elevated plasma ACE levels is secondary to a linkage disequilibrium (LD) effect.4 We recently showed this to be the case for the association of the I/D polymorphism with essential hypertension as well.5,6 To verify the effect of a gene it is important to genotype several single nucleotide polymorphisms (SNPs) across the gene, study the structure of the haplotype block and look for associations with the haplotypes, which considerably increases the power of a case-control study. Moreover, improved study designs such as sib pair and TDT-trios are formidable tools for teasing out the genetic architecture of complex disorders. Elaborate algorithms exploring the combinatorial effects of SNPs in several candidate genes across the genome have recently become available and can add power to the analysis.7 Using novel study designs entire pathways, such as the renin-angiotensin system (RAS), can be explored in complex disorders such as SAH. This is important as some components of RAS increase angiotensin II formation whereas others decrease it.8 It is also worthy to collect details of disease characteristics which may serve as covariates in subsequent allele, genotype and haplotype analysis. The study design can be enhanced by including intermediate phenotypes in the study.9 Recently, genome-wide SNP analysis using microarrays has brought linkage analysis for complex disorders within the realm of reality,10 provided suitable pedigrees are available.

Without such elaborate efforts it will be difficult to meaningfully interpret results of genetic association studies for complex disorders. Studies of human polymorphisms had a modest presence in 1980 with just over 100 publications. After the explosive entry of ACE I/D in 1992 and ApoE e-4 in 1993 in the complex disease arena, there was an exponential rise in the studies of human polymorphisms, most being association studies. Approximately 3500 such studies are now published annually in indexed journals. Making sense of such a large number of studies, several presenting contradictory findings, is a colossal task. Unless well-determined guidelines are followed, and robust study designs and approximate sample sizes are used, many resources will go to waste. Also, a majority of the genome will remain unexplored in most disorders, and only a few loci will be investigated repeatedly with no additional information gained. It is important that we realize the significance of well-planned genetic association studies aimed at stroke and associated complex disorders. In light of this, the National Institutes of Health funded Siblings with Ischemic Stroke Study is much appreciated.11 Additionally, a Stroke Consortium could be made which would allow the use of DNA and other resources to investigators on approval of a proposal in a similar fashion as the Framingham Heart Study.12 We must act before it is too late.

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2. Kermani et al.2 showed that the I/D polymorphism is not functional and its association with elevated plasma ACE levels is secondary to a linkage disequilibrium (LD) effect.4
3. Using novel study designs entire pathways, such as the renin-angiotensin system (RAS), can be explored in complex disorders such as SAH. This is important as some components of RAS increase angiotensin II formation whereas others decrease it.8
Response:

We thank Dr Saeed for his letter emphasizing a very important aspect concerning the design of genetic studies, i.e., the calculation of the power of a study and the number of patients included in the control and case groups needed to provide replicable results. Interestingly, while analyzing the studies that assess the significance of different polymorphisms in a variety of stroke etiologies, which have been published in Stroke during the last five years, we found that this aspect of methodology was addressed only in a few cases. In general, the previous authors published in Stroke calculated the required number of cases and controls to achieve the power of 80% ($P = 0.05$). This was based on allele or genotype frequencies in the populations to achieve a given minimum odds ratios, the relative risk by allele, or the proportion of the studied alleles in cases and controls. Other journals, such as Neurology and Human Genetics have already published the guidelines for genetic association studies in humans. We feel it would be reasonable to prepare such guidelines for publication in Stroke. The information regarding the power of a study should be provided, in a uniform fashion, within the methodology section of all articles related to this topic.

A very important aspect in the design of genetic association studies (or any clinical study, for that matter) is the ability to generate significant and replicable results while keeping within budget constraints. It is well known that the population frequency of the II genotype, and not I allele, of the angiotensin-converting enzyme (ACE) gene is approximately 25% (23.7% in English patients and 23.4% in our controls). Bearing this in mind, we calculated that having 90 patients with aneurysmal subarachnoid hemorrhage and 128 controls, we would be able to obtain statistically significant differences between the groups if the odds ratio was $> 2.5$, assuming the study's achievable power to be 80%, and $P = 0.05$. In our study, type I error (probability of rejecting true H0 hypothesis) was 0.0001, which is very low.

It is commonly known that ACE gene insertion/deletion polymorphism, because it is an intronic marker, may be functionally neutral, but may be in linkage disequilibrium with other (functional) mutations within ACE or another gene. The genetic association studies, such as we performed, generally answer the question as to whether the target allele or genotype remain risk factors for the disease, but they do not answer the question, does the causal relationship exist between them? We can only speculate therefore whether such a causal relationship exists. We have already planned to expand our studies of the ACE gene, examining several single nucleotide polymorphisms across the gene, and calculating association with haplotype(s). We agree with Dr Saeed that introducing genome-wide single nucleotide polymorphism analysis using microarrays is an approach that will ultimately enable study of complex disease mechanisms in greater detail.

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Letters to the Editor 2753


Is Perimesencephalic Nonaneurysmal Hemorrhage of Venous Origin?

To the Editor:

We read with great interest the article by van der Schaaf and colleagues in the July 2004 issue of Stroke in which the authors sought to assess the contribution of venous drainage patterns in the etiology of perimesencephalic hemorrhage (PMH). PMH, defined by a negative 4-vessel angiographic study as bleeding in the cisterns around the midbrain, is associated with an excellent outcome. Using computed tomography angiography, van der Schaaf and colleagues found that patients with PMH had primitive venous drainage directly into dural sinuses instead of via the vein of Galen, as with patients having aneurysmal subarachnoid hemorrhage. They also observed that the side of the PMH related to the side of the primitive drainage. They concluded that these results support the venous origin of PMH. Although van der Schaaf and colleagues found differences in the venous drainage of these patients, they neglected to present direct evidence showing that primitive venous drainage led to hemorrhage around the midbrain. They speculated that direct venous pressure from the dural sinus might cause the rupture of the perimesencephalic veins. However, their speculation is not substantiated because other cerebral veins with direct connection to the dural sinus never rupture under normal circumstances. Moreover, if this variation of venous drainage were a cause of bleeding, it would have a high chance of rebleeding in the follow-up because venous drainage is not easily changed.

We would like to bring to the authors' attention our study of PMH in which small bulges on the basilar artery were found in 2 patients by 3D rotational angiography. These lesions were thought to be intramural hematomas due to arterial dissection and to be responsible for the hemorrhage in each case. This finding matches the distribution of the hematomas, and the spontaneous healing of the dissection could explain the absence of rebleeding. The small bulges showed spontaneous resolution, but they were too small to be detected by computed tomography angiography, such as that employed by van der Schaaf and colleagues. Therefore, we would like to emphasize that some of the PMH were of arterial origin. Nonetheless, the study by van der Schaaf and colleagues, which focuses on venous drainage, provides an

important contribution to the literature. We congratulate the authors on their careful observations.

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Venous Drainage in Perimesencephalic Hemorrhage: Is Perimesencephalic Nonaneurysmal Hemorrhage of Venous Origin?

Response:

We appreciate the interest of Dr Matsumaru and colleagues in our article on the venous drainage patterns in the etiology of perimesencephalic hemorrhage (PMH).1

Dr Matsumaru focuses our attention on their observation in 2 patients with a perimesencephalic pattern of hemorrhage, who had small bulges on the basilar artery that disappeared on follow-up imaging. These bulges were supposed to be intramural hematomas due to arterial dissection, and were not visible on standard DSA but only on the 3-D angiography.2 The authors emphasized that some of the patients with a PMH might have had an arterial origin.

We are aware that in patients with a perimesencephalic pattern of hemorrhage, the cause is of arterial (vertebrobasilar) origin in 5% of the cases.3 We also agree that an abnormal venous drainage is not the definitive cause in all patients with a PMH. However in patients with a unilateral primitive variant, the blood draining is not the definitive cause in all patients with a PMH.5% of the cases.3 We also agree that an abnormal venous drainage in patients with subarachnoid hemorrhage, but without an aneurysm on angiography.7 In most patients with subarachnoid hemorrhage from intracranial dissections, the pattern of hemorrhage is not confined to the perimesencephalic cisterns, but comparable to an aneurysmal pattern of hemorrhage. However, intracranial dissections leading to a subarachnoid hemorrhage have a 30% risk of rebleeding.8 These characteristics are unlike those from PMH, and we do not think that arterial dissections are a frequent cause of PMH. The relatively benign symptoms at onset, the clinical course without rebleeding or secondary ischemia, and the localized pattern of hemorrhage favor a venous source in the majority of these patients. The observation of Dr Matsumaru et al offers an interesting vision in the etiology of PMH, which may hold true for some patients with a perimesencephalic pattern of hemorrhage. The higher resolution of the MS CT scanners and 3D angiography will provide more insight on this finding in the future.

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Contrast Ultrasound Techniques in the Detection and Quantification of Patent Foramen Ovale: Myth Versus Reality

To the Editor:

I read with great interest the study of Anzola et al. The authors declared contrast-enhanced transcranial Doppler (ce-TCD) as the ideal tool to follow up patients after interventional device closure.1

Data show a high correlation between ce-TCD and contrast enhanced transesophageal echocardiography (cTEE) which is the current “gold standard” for detection of a right-to-left shunt through a patent foramen ovale (PFO), although there is a highly reported interobserver and intraobserver variability of the latter.2 Furthermore, there are major methodological limitations of these techniques that need to be addressed.

The numbers of microbubbles reaching the brain can be quantified by ce-TCD, however the therapeutic impact of this information is unknown. There is no prospective study (neither by ce-TCD nor by cTEE) demonstrating that the amount of contrast shunting has any relevance for the outcome of these patients.2,3 We have previously shown that the amount of contrast shunting does not correlate with the size of the PFO measured by 2-dimensional TEE or invasively by balloon sizing.4

Devuyyst and colleagues reported that the amount of right-to-left contrast shunting through a PFO measured my ce-TCD mainly depends on strain rate and duration of the Valsalva maneuver.5 As Anzola et al did not perform the ce-TCD follow up under these controlled conditions, 2 contrast studies may lead to different results in the same patient even if the PFO would not have been closed.

It has been shown previously that ce-TCD with saline contrast can remain positive, even without difference in the intensity, after complete occlusion of anatomical arteriovenous malformations.6 According to recent findings one must accept that there is no rigid diagnostic time window for differentiation between intra-arterial and intrapulmonary shunts.7–10 And there is evidence that physiological arteriovenous intrapulmonary shunts do exist in most healthy humans.11 As we know so far, shunting through this dynamic vascular network is influenced on a variety of patient dependent and methodological reasons like cardiac output, blood pressure, or drugs.11–13

Before and after PFO-closure we recommend, irrespective of the results of a ce-TCD, to perform a TEE examination, as it provides direct anatomic information regarding the site and nature of the shunt and regarding the device to exclude a thrombus formation.14

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Response:

Dr Schuchlenz’s letter seems to put a special emphasis on the lack of specificity of contrast-enhanced transcranial Doppler (ce-TCD) in differentiating the source of a right-to-left shunt (RLS), namely patent foramen ovale (PFO) versus pulmonary fistulas. In our opinion, however, instead of showing limitation, this is an argument in favor of transcranial Doppler (TCD) as an ideal tool for screening and follow-up purposes inasmuch as paradoxical embolism is suspected responsible for otherwise cryptogenic strokes. It is commonly recognized that TCD detects more RLSs than transesophageal echocardiography (TEE), not only because TEE may miss pulmonary fistulas but also because even in cases of PFO, the patient may be unable to perform a Valsalva strain valid enough to divert bubbles from the right to the left atrium across the PFO.1 This raises some doubt on the concept of TEE as the gold standard for PFO detection.

There are at least 2 studies that have shown a relationship between the amount of RLS as assessed with TCD and stroke occurrence or relapse.1–3

The ce-TCD test was always performed in controlled conditions following the recommendations of the Consensus of Venice4 and the strength of Valsalva was regulated so as to obtain a reduction of at least 20% of the spectral amplitude. For these reasons we believe that the bubble load assessed in the middle cerebral arteries at follow-up testing truly reflected the degree of residual shunt.5

The fact that TCD (vs TEE) remain positive after apparently successful embolization of a pulmonary arteriovenous malformation (AVM) in a patient with Rendu-Osler disease, as is reported in Yeung et al is not surprising for at least 2 reasons. First, the embolization of a macroscopic pulmonary AVM may not abolish the local RLS. Second, Rendu-Osler disease entails the presence of multiple microscopic AVMs that can escape angiography but nonetheless bring about a cumulatively significant RLS.
Physiological intrapulmonary shunts are activated after prolonged strenuous exercise whereas ce-TCD requires a simple Valsalva strain for a few seconds.

None of the patients in our series was under the effect of drugs likely to affect intrapulmonary shunts. Our policy is to perform TEE after TCD demonstrates RLS to confirm the intracardiac site before PFO closure and to repeat it postprocedurally only in those patients in whom TCD has shown a significant residual shunt or in those who relapse after successful closure. Routine use of TEE to detect thrombus formation in the left atrium does not seem justified in asymptomatic patients given the very low absolute incidence of thrombus formation with the more recent devices.

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Letters to the Editor
White Matter Hyperintensities: Pearls and Pitfalls in Interpretation of MRI Abnormalities

To the Editor:

Atwood et al acknowledge that the pathophysiology of white matter hyperintensities (WMH) is uncertain and underscore the possibility of ischemic etiology, especially in the elderly. These authors regard WMH as an excellent marker of brain aging and emphasize their heritability in patients with negative correlation with cerebrovascular brain injury.

WMH are neither age-specific nor generally heritable, having been found in both sexes in hypertensive encephalopathy, perinatal eclampsia, migraine, and therapy with cyclosporin, interferon-α, and tacrolimus. Kruit and coworkers found WMH in MRI only in women migraine patients, which likely reflects an investigational artifact. Atwood et al did not exclude hypertensive and migraine patients from their analysis, which prominently confounds their interpretation of WMH as indicative of brain aging.

Instead of being a result of cerebrovascular disease, WMH probably result from intense, but self-limited cerebral hyperperfusion. I propose that WMH are markers of transient breakdown of the blood–brain barrier rather than aging.

The heritability of WMH volumes is an intriguing feature. The decline in heritability estimates after age 60 indicates the nongenetic nature of this observation. Another indicator of the nongenetic nature of WMH is the absence of correlation with aging in women despite higher heritability. Migraine is more prevalent in women than in men, from approximately age 12 to 14. Breakdown of the aging-marker hypothesis for WMH in women may relate to migraine headaches. Finally, heritability of WMH may relate more to heritability of hypertension or migraine or both. In the absence of any link to cerebrovascular disease, the menopause probably has no independent bearing on WMH. Spontaneous resolution likely underlies significantly smaller WMH volumes at younger age, especially in women, in which cohort the highest prevalence of migraine can be expected. These authors also hope to establish a genetic link between WMH and silent brain infarctions. Unless the resolution or otherwise of WMH is established prospectively, it is premature to link this MRI finding with cerebrovascular ischemic disease. Cross-sectional studies of WMH cannot establish vascular-related genetic influences, as has been suggested. Assumption of the genetic model for WMH is probably incorrect.

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Response:

In his letter to the editor, Dr Gupta argues that white matter hyperintensities (WMH) are neither age-specific nor heritable. As evidence in support of this argument, he expands on an already lengthy list of causes for WMH in order to underscore the nonspecific nature of these phenomena. He then focuses on the distribution of WMH in migraine patients to support his hypothesis that WMH represent transient breakdown of the blood–brain barrier rather than the aging process per se. Finally, Dr Gupta concludes that our data do not support the heritability of WMH.

In response to his observations that WMH should be considered nonspecific, we agree completely. Many diseases lead to WMH. We must note, however, that most of the causes identified by Dr Gupta are extremely rare in a community-based population such as the Framingham Heart Study, suggesting that the overall influence of these diseases is likely to be extremely low. Regarding his hypothesis that WMH reflect a breakdown of the blood–brain barrier, there is ample evidence to support this, particularly in multiple sclerosis; but again, the prevalence of this disease in a healthy population is so low as to be negligible, and all known subjects with multiple sclerosis or other neurological diseases affecting white matter were systematically excluded. Dr Gupta does note that transient breakdown of the blood–brain barrier may be common to migraine, but this would not explain our findings as migraine declines in prevalence with advancing age. In addition, we note that age-related increases in WMH volume are one of the most consistent findings in studies that limit analysis to extremely healthy individuals.1–4 From these data, we conclude that age, in fact, does relate to WMH volumes across both age and gender differences. Dr Gupta’s second argument for heritability reflects an apparent misunderstanding of WMH.

Dr Gupta also argues that our data do not conclusively show heritability of WMH. As evidence for this, he notes the decline in heritability after age 60. He further concludes that our data would not explain our findings as migraine declines in prevalence with advancing age. In addition, we note that age-related increases in WMH volume are one of the most consistent findings in studies that limit analysis to extremely healthy individuals.1–4 From these data, we conclude that age, in fact, does relate to WMH volumes for the general population. In the manuscript, however, we do caution that age-related cardiovascular diseases such as systolic hypertension are also likely to contribute to the extent of WMH,5 leading us to suggest that WMH may serve as a phenotypic marker for both aging and disease.

Nicotinamide Attenuates Focal Ischemic Brain Injury: Meta-Analysis or Mechanism of Protection

To the Editor:

We read with great interest the recent article by Macleod et al.1 They have shown a highly significant neuroprotective effect of nicotinamide on experimental stroke using the technique of systematic review or meta-analysis. The message is clear, and we believe this approach is useful and fairly robust in terms of statistical power. However, I would like to point out a possible pitfall of their overview. Nicotinamide, vitamin B3, is the precursor of nicotinamide adenine dinucleotide (NAD⁺), and it may act as a poly(ADP-ribose) polymerase (PARP) inhibitor. When ischemia-induced DNA strand breakage is too extensive, the overactivation of PARP may lead to intracellular NAD⁺ depletion and subsequent secondary energy failure (ie, ATP depletion).2 ATP levels can be dramatically increased following ischemia-reperfusion by nicotinamide.3 This scenario is generally accepted to explain the neuroprotective action of nicotinamide. However, it is also well known that high doses of nicotinamide (250 mg/kg in our case) increase regional cerebral blood flow, and many studies have ignored cerebral blood flow. Therefore, we cannot jump into a conclusion that nicotinamide is a pure neuroprotectant independent of vasodilative action. If one does not consider divergent mechanisms of the beneficial effects of a candidate drug, such a promising drug based on animal experiments may fail to work under clinical settings, even though pooling of animal data reveals undoubtedly obvious neuroprotection.
Dramatic early and good results were reported in the early (42% early magnesium infusion in ischemic or hemorrhagic stroke in might be used. research and to develop a standard set of methodologies which

tional collaborative approach to establish priorities for future

provide further insights both into the limits to the efficacy of individual drugs and into more generic determinants of outcome incorrectely attributed to some other drug property.

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give a more precise estimate of the effect of a drug on CBF. This

where data are available the same technique could be used to

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Changes in CBF. However, power calculations for such compar-
tions are regulated by active blood–brain barrier transport.2,3

barrier, in both animals and in humans.1 Brain magnesium concen-

provides substantial support for clinical stroke trials in humans.

In fact, the basic neuropharmacology of magnesium sulfate

strongly disagree with his assessment of the pertinent literature.

viewed critically and cautiously.

The adaptive nature of hypomagnesemia, seen in a wide

variety of clinical conditions and circumstances, including hos-
pitalization and diabetes mellitus, is not generally appreciat-
ed.4,5,6 Magnesium impairs cardiac pump function, a physiolog-

cal limitation to rapid therapeutic supplementation in acute

myocardial infarction or congestive heart failure.5 With a mean

patient age of 74 years (range 44 to 92 years) in this trial,1 the

negative inotropic effect of magnesium infusion5 can significan-
tly impair cardiac output and cranial perfusion pressure in this

presbyocardiac cohort. Informed consent from potential patients

for clinical trials involving magnesium supplementation must

include pharmacokinetic information about magnesium and

BBB. Absence of serious side effects1 does not by itself support

use of any neuroprotective agent. No large-scale trial of magne-
sium supplementation in stroke, in the field or in the hospital,

should be envisaged without presenting this facet of magnesium

pharmacology to the approving institutional review board.

Vinod K. Gupta, MD

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Response:
The comments of Yao and colleagues serve to further illustrate
the potential uses of systematic review and meta-analysis in the
context of the interpretation of data from animal studies in stroke.

Studies in focal cerebral ischemia may report measurement of
cerebral blood flow (CBF). In most cases, they find no signifi-
cant differences between control and treated groups, and use this
to infer that any protective effect seen is not a consequence of
changes in CBF. However, power calculations for such compar-
sions are seldom if ever reported; it may be that a true difference
in CBF is missed because studies are underpowered for this
comparison (ie, there is a type II or “false negative” error).

Analyses such as ours can illuminate the impact of study
quality and study design on the estimate of efficacy. Equally,
where data are available the same technique could be used to

give a more precise estimate of the effect of a drug on CBF. This

would reduce the probability of a type II error, and thereby
reduce the risk that protection due to an effect on CBF might be
incorrectly attributed to some other drug property.

It is likely that systematic review and meta-analysis can
provide further insights both into the limits to the efficacy of
individual drugs and into more generic determinants of outcome
in experimental stroke. We are currently developing an interna-
tional collaborative approach to establish priorities for future
research and to develop a standard set of methodologies which
might be used.

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Intravenous Magnesium for Neuroprotection
in Acute Stroke: Clinical Hope Versus Basic Neuropharmacology

To the Editor:

Saver and colleagues investigated the neuroprotective effect of
early magnesium infusion in ischemic or hemorrhagic stroke in the
field; three quarters of the infarct cohort were treated within
2 hours of onset, and nearly one-third within 1 hour of onset.1
Dramatic early and good results were reported in the early (42% of <2-hour infarct patients) and 90-day global functional outcomes (69% of all patients and 75% of <2 hour infarct patients), respectively. These authors recommend large-scale trials with field initiation of magnesium for early neuroprotection in stroke.

An essential prerequisite for any pharmacological agent to offer significant brain neuronal protection during strokes is its ability to freely cross the blood–brain barrier (BBB). Transport of magnesium from blood to cerebrospinal fluid across BBB is limited in normal humans; intravenous administration of magnesium sulfate does not increase cerebrospinal fluid magnesium concentration.2 Orally or intravenously administered magnesium sulfate cannot affect brain neuronal function.3 Acute stroke is a hemodynamically highly labile clinical condition and impressive functional outcomes with magnesium supplementation should be viewed critically and cautiously.

The adaptive nature of hypomagnesemia, seen in a wide variety of clinical conditions and circumstances, including hospitalization and diabetes mellitus, is not generally appreciated.4,5,6 Magnesium impairs cardiac pump function, a physiological limitation to rapid therapeutic supplementation in acute myocardial infarction or congestive heart failure.5 With a mean patient age of 74 years (range 44 to 92 years) in this trial,1 the negative inotropic effect of magnesium infusion5 can significantly impair cardiac output and cranial perfusion pressure in this presbyocardiac cohort. Informed consent from potential patients for clinical trials involving magnesium supplementation must include pharmacokinetic information about magnesium and BBB. Absence of serious side effects1 does not by itself support use of any neuroprotective agent. No large-scale trial of magnesium supplementation in stroke, in the field or in the hospital, should be envisaged without presenting this facet of magnesium pharmacology to the approving institutional review board.

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Intravenous Magnesium for Neuroprotection in Acute Stroke: Neuropharmacology Supports Clinical Hope

Response:

We thank Dr Gupta for his interest in our study, but we
strongly disagree with his assessment of the pertinent literature. In
fact, the basic neuropharmacology of magnesium sulfate
provides substantial support for clinical stroke trials in humans.

Several studies show that magnesium does cross the blood–brain
barrier, in both animals and in humans.1 Brain magnesium concen-
trations are regulated by active blood–brain barrier transport,2,3
Cerebrospinal fluid magnesium concentration increases by 20% to 25% in response to doubling of the serum concentration, and peaks around 4 hours after parenteral administration.3,4 While this overall increase in cerebrospinal fluid magnesium concentration is modest, magnesium concentration is selectively substantially increased in regions of pathology, including focal ischemia and seizures.6,7
It is also well known that the mild negative inotropic effect of magnesium sulfate is offset by its lowering of peripheral vascular resistance, resulting in no clinically substantial impairment in cardiac pump function. Several physiological studies suggest that magnesium increases cardiac output. Even in patients experiencing active myocardial ischemia, magnesium sulfate showed only a very small increase in the incidence of cardiogenic shock or congestive heart failure in the large ISIS-4 trial, and no adverse effect on cardiac pump function was reported in the more recent MAGIC clinical trial. Most saliently, among stroke patients in the phase 3 IMAGES trial, there was no excess of cardiac events related to administration of magnesium sulfate.

In addition, magnesium sulfate is a potent cerebral vasodilator, in part due to calcium channel antagonism at vascular smooth muscle cells and possibly effects on myosin-binding proteins that regulate contraction. Consequently, magnesium sulfate typically increases, rather than decreases, cerebral perfusion.

Magnesium sulfate has been demonstrated to decrease infarct volume in multiple animal models of stroke, has numerous identified beneficial neuroprotective and vascular effects, is already known to be efficacious in treating in humans a condition characterized by altered cerebral blood flow ( eclampsia), and has shown a potential signal of efficacy when administered early in part due to calcium channel antagonism at vascular smooth muscle cells and possibly effects on myosin-binding proteins that regulate contraction. Consequently, magnesium sulfate typically increases, rather than decreases, cerebral perfusion.

It is also well known that the mild negative inotropic effect of magnesium sulfate is offset by its lowering of peripheral vascular resistance, resulting in no clinically substantial impairment in cardiac pump function. Several physiological studies suggest that magnesium increases cardiac output. Even in patients experiencing active myocardial ischemia, magnesium sulfate showed only a very small increase in the incidence of cardiogenic shock or congestive heart failure in the large ISIS-4 trial, and no adverse effect on cardiac pump function was reported in the more recent MAGIC clinical trial. Most saliently, among stroke patients in the phase 3 IMAGES trial, there was no excess of cardiac events related to administration of magnesium sulfate.

In addition, magnesium sulfate is a potent cerebral vasodilator, in part due to calcium channel antagonism at vascular smooth muscle cells and possibly effects on myosin-binding proteins that regulate contraction. Consequently, magnesium sulfate typically increases, rather than decreases, cerebral perfusion.

Magnesium sulfate has been demonstrated to reduce infarct volume in multiple animal models of stroke, has numerous identified beneficial neuroprotective and vascular effects, is already known to be efficacious in treating in humans a condition characterized by altered cerebral blood flow ( eclampsia), and has shown a potential signal of efficacy when administered early after stroke onset (within 3 hour subgroup) in a randomized clinical trial. Further trials of magnesium sulfate in early time epochs in acute stroke are well-supported by preclinical and clinical neuropharmacology.

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Letters to the Editor

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Aspirin for Stroke Prevention Taken in the Evening?

To the Editor:

We read the article by Yip et al1 with great interest, in which they demonstrated that platelet activation significantly increases in acute ischemic stroke and substantially decreases thereafter. The lesser long-term pharmacodynamic potency of aspirin relative to clopidogrel raises the prospect of the need for more effective antiplatelet agents or a synergistic combination therapy for stroke prevention in the future.1 Their results are very impressive and raise some ideas, particularly associated with the prevention’s procedures.

According to World Health Organization data, in 1996 4.6 million people in the world died because of cerebrovascular manifestation of atherothrombosis.2 In Hungary, nearly 18 000 people suffer stroke every year, half of whom die within a year. In cases of both cardiovascular and cerebrovascular diseases the significant decrease of morbidity/mortality can only be achieved by increasing the effect of prevention. A major form of secondary prevention is the administration of drugs inhibiting the aggregation of platelets.

Aspirin is one of the most common, useful, and inexpensive tools for prevention. The effect of aspirin on platelets is irreversible lasting for the duration of the platelet’s life span (~10 days). Aspirin-mediated inhibition of platelet function occurs within 60 minutes of ingestion.

The incidence of stroke assessed by onset of clinical symptoms exhibits a marked circadian variation with a peak period during the morning. Stroke usually occurs unexpectedly and more frequently in early morning hours (between 5 to 12 pm; see Figure) when the aggregability of thrombocytes is higher.4

The September 2003 edition of Endovascular Today devoted a section to an update on CAS.1–6 Three of 4 clinical authors were chief medical editors of the journal and the other author was on the Editorial Advisory Board. Of these 4 authors, three are consultants to medical device companies (Cordis, Medtronic, and Guidant).

Are the reported results of CAS truly unbiased? Many of the leading investigators are paid consultants of medical device manufacturers. As a specialist in vascular disease, and specifically a vascular surgeon, I perform CEAs. I also perform stent procedures in most peripheral arteries where indicated and stent graft repairs of abdominal aortic aneurysms. These procedures are effective and offer patients potentially less morbid alternatives to open operations. On the other hand, CAS offers little advantage over CEA, a minimally invasive procedure itself. Theoretically, if one considers the pathology being treated and the sensitivity of brain tissue to a lack of blood supply, CAS is difficult to justify. If it is truly safer, I will perform the procedure like others in my specialty. Nonetheless, in such a potentially high-risk area, the public deserves meticulous analysis by unbiased investigators who have no financial tie to the results.

Relation of plasma serum levels of salicylacid and the daily distribution of the occurrence of acute ischemic stroke. Vertical bar graphs represent daily distribution of incidence of ischemic stroke.4 Line diagrams show serum level of salicylacid in μg/mL taken at 10:00 PM (dotted line) and at 8:00 AM (solid line). The maximum plasma concentration of salicylacid occurs 3.5 to 4.0 hours after intake.5 N=total number of patients involved in analysis, SA=salicylacid.
Increased platelet aggregation in the morning and perhaps an upright posture may account at least in part for the observed circadian variation of the manifestation of stroke.

For prevention patients usually take aspirin in the morning. The treatment regimen is 1 tablet (100 mg) per day to be swallowed without chewing at least 30 minutes before breakfast. The relation of occurrence of stroke and the change of aspirin’s plasma level taking in the morning is demonstrated in the Figure. It is obvious that highest plasma level of the drug occurs after the morning peak-incidence of the thromboembolic event, suggesting lower prophylactic effect of aspirin. Furthermore, this treatment regimen has its highest protective effect during the day, when, synergistically, normal physical activity exerts a protective action on thromboembolic processes. However, this method of daily aspirin administration has its lowest protective value against cardiovascular events during the night and early morning, when the lack of physical activity further augments the cascade of hemorheological events favoring platelet aggregation and subsequent ischemia. In contrast, highest plasma level of aspirin taken late evening (10:00 PM) would be reached prior to the peak-incidence of thromboembolic disorders. We are confident that this time shift in the administration of aspirin would fit better in the circadian scheme of the occurrence of stroke, thus resulting in a significantly more effective prevention. To prove the viability of this concept we propose to set up international, randomized, multicenter studies.

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Response:
Platelets play a key role in the pathogenesis of atherosclerosis and arterial thrombosis, which are the major contributors to the development of ischemic stroke and acute coronary syndrome.1–3 Antiplatelet therapy is now a mainstay of primary and secondary prevention of death, myocardial infarction, and stroke in various categories of patients.4–6 Previous studies have demonstrated the ischemic stroke to display a diurnal variation with a higher frequency in the early hours of the morning.7,8 Although exactly why this circadian variation occurs remains uncertain, circadian variation of fibrinolysis, platelet aggregability, and arterial blood pressure, with their peak value in the morning, have been suggested to play a key role in this phenomenon.7–9 Given the safety, widespread availability, and minimal cost of aspirin therapy, aspirin is one of the most common antiplatelet agents for secondary prevention following an ischemic stroke.10 Aspirin is rapidly absorbed in the upper gastrointestinal tract and results in a measurable inhibition of the platelet function within 60 minutes.11 Additionally, a pharmacokinetic study has demonstrated that the peak serum level of aspirin is generally detected within 3.5 to 4.0 hours following ingestion.12 While considering both the circadian variation of a stroke and the particular pharmacodynamic effect of aspirin, Bodis and colleagues conjectured that taking aspirin in the morning after breakfast, as traditionally recommended, would offer a lower protective effect on recurrent stroke. They proposed that aspirin taken in the late evening, i.e., around 10:00 PM, would benefit patients more than aspirin taken during the traditional administration time. To prove the viability of this hypothesis, they propose to set up international, randomized, multicenter studies.

We read with great interest regarding their hypothesis and opinion. However, 3 issues should be considered before conducting trials to test their hypothesis. First, if taking aspirin in the late evening could provide additional benefit than taking aspirin in the morning, a concern may arise over whether taking aspirin (100 mg) twice daily might be more protective to patients than daily administration. Second, owing to the benefits of aspirin in treating acute ischemic stroke,10 acute coronary syndrome,3 and in the secondary prevention for cardiovascular and cerebrovascular diseases,2 numerous studies have attempted to evaluate aspirin as a primary prevention strategy.5,6,13 While the results regarding the role of aspirin on the primary prevention of cardiovascular mortality remains inconsistent,4,11,14 aspirin does not appear to significantly affect the primary prevention of a stroke.6,14 These observations motivate the search for a more effective antiplatelet agent other than aspirin for primary stroke prevention in the future. Third, several randomized trials3,5,15 have been conducted to determine the extent of reduction in terms of cardiovascular outcomes by different antiplatelet therapies in patients at risk of ischemic stroke. Clopidogrel, a new thienopyridine derivative that is chemically related to ticlopidine, blocks activation of platelets by selectively and irreversibly inhibiting the binding of adenosine diphosphate (ADP) to its receptor on platelets, subsequently inhibiting the ADP-dependent activation of the Gp IIb–IIIa complex, the major receptor for fibrinogen binding on platelet surface.5 A related study has demonstrated that clopidogrel is more effective than aspirin in reducing the combined risk of ischemic stroke, myocardial infarction, or vascular death.5,16 Our study has provided basic evidence that clopidogrel is more potent than aspirin in suppressing platelet activation after ischemic stroke, and, therefore, further strengthens the conclusions of previous studies.3,5,15 Although testing the hypothesis whether aspirin administered in late evening (at 10:00 PM) would offer greater benefit to patients would be more interesting, identifying a more effective antiplatelet agent for primary or secondary prevention of ischemic stroke would be more meaningful.

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**Predicting rtPA Associated ICH in Acute Stroke**

*To the Editor:*

I read with interest the study by Trouillas et al from Lyon, France,1 describing a plasma marker associated with parenchymal brain hemorrhage in patients treated with intravenous recombiant tissue plasminogen activator (rtPA) for acute stroke. Unusually high D-dimer levels measured at 2 hours after beginning rtPA were associated with increased risk of parenchymal hemorrhage within 24 hours. This is useful information that could be translated to patients treated elsewhere with a similar protocol. However, the currently adopted protocol for intravenous rtPA treatment of acute stroke in the United States and many other countries is according to the American Stroke Association (ASA)2 and the American Academy of Neurology (AAN)3 guidelines, and these guidelines differ in 2 important ways from the protocol used in Lyon. I would like to point out that because of these differences, the findings from Lyon may not translate to centers that follow the ASA/AAN guidelines.

First, the ASA/AAN guidelines do not recommend treatment beyond 3 hours after symptom onset, compared with up to 7 hours in Lyon. The mean delay to starting intravenous rtPA in a considerable proportion of the patients in Lyon (exact proportion not stated).4 This postthrombolytic increase of FDP at 2 hours (early fibrinogen degradation coagulopathy) was correlated with a fibrinogen decrease, indicating an impact of rtPA, at least partial, on circulating fibrinogen. It may be possible that D-dimers, which have a different biological significance, would not be predictive of early parenchymal hematoma. However, this measurement is now included in our prospective hemostasis evaluation.

Second, the Lyon protocol4 was designed in 1993, before the NINDS rtPA Study5 was published. At that time, the dose of rtPA, the window of administration, and the anticoagulant regimens were not standardized. However, the dose chosen was remarkably similar to that of the NINDS study (0.8 mg/kg). After the publication of the NINDS study results, we decided to continue the trial up to 200 patients, because our protocol was the unique opportunity to know what gave a slightly lower rtPA dose with a longer infusion time (90 minutes), a wider time window (7 hours), and precise postthrombolytic heparin regimens. In the cohort of 157 patients with hemostasis screening,1 the rate of symptomatic hemorrhage (3.8%) was lower than in part 1 (6%). This postthrombolytic fibrinogen degradation coagulopathy does not seem to be influenced by heparin. In our cohort, only 20.3% of patients had a regimen with immediate postthrombolytic unfractionated heparin. In several myocardial infarction trials including immediate use of intravenous heparin, an early FDP increase took place and was demonstrated to be correlated with bleeding complications, general or cerebral.6–11

Thus, elevated D-dimer levels in acute stroke patients treated with intravenous rtPA according to the ASA/AAN guidelines may not be predictive of parenchymal hemorrhage, as they are in patients treated with the Lyon study protocol. It would be useful to validate the findings from Lyon in patients treated within the ASA/AAN guidelines.

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Thus, there is a chance that early fibrinogen degradation coagulopathy, predictive of early parenchymal hematomas, may be confirmed in rtPA protocols following the ASA/AAN guidelines, without heparin during the 24 first hours, as well as in intraarterial protocols like PROACT II using postthrombolytic heparin. Another answer may be given by the FRALYSE study, ongoing in France: this randomized, assessment blinded study, compares arms with the NINDS and Lyon protocols, with hemostasis and clinical parameters.

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ACE and Subarachnoid Hemorrhage: Strategies for Genetics of Stroke
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Stroke. 2004;35:2752-2753
doi: 10.1161/01.STR.0000149991.62457.40
Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0039-2499. Online ISSN: 1524-4628

The online version of this article, along with updated information and services, is located on the
World Wide Web at:
http://stroke.ahajournals.org/content/35/12/2752

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