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**Cerebral Venous Sinus Thrombosis: Prevention of Recurrent Thromboembolism**

B.F.L. van Nuenen, M. Munneke and B.R. Bloem

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# Letters to the Editor

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## Protein Z Levels, Protein Z G79A Polymorphism, and Prothrombotic Conditions

*To the Editor:*

We read with great interest the article on protein Z gene polymorphisms, protein Z concentrations, and ischemic stroke published by Staton et al.[1] The authors examine protein Z concentrations and 2 common polymorphisms of the protein Z gene in patients with a first-ever ischemic stroke and conclude that high levels of protein Z are prothrombotic and that the 79AA genotype of the G79A polymorphism is protective for the ischemic stroke.

The conclusions drawn by Staton et al seem questionable because of several flaws.

First, the hypothesis that high levels of protein Z are prothrombotic is, to date, not supported by the pathophysiology, because the role of protein Z has been demonstrated to be the inhibition of activated factor X through the formation of a complex with protein Z–dependent protease inhibitor. Consequently, low levels of protein Z are expected to be prothrombotic. In line with this theory, the majority of the studies in this field have reported low levels of protein Z in association with prothrombotic conditions. Actually, only 2 studies have reported that high levels of protein Z are prothrombotic, but methodological biases are detectable. In the former, the investigators modified the commercial assay and used a normal plasma pool.[2] In the latter, the control group included subjects with a previous vascular event.[3]

Second, Staton et al reported lower levels of protein Z plasma in controls with respect to all the other case-control studies present in the literature, including reports with a greater number of subjects analyzed. This might be attributable to the presence of subjects with a previous vascular event (not better clarified) included in the control group. Actually, patients with vascular diseases have been reported to have low levels of protein Z.[4]

Third, the significant result of the 79A allele as a protective factor for the ischemic stroke has been performed by pooling data from a previous study. Actually, a pooled analysis in case-control studies, especially for the evaluation of genetic polymorphisms, is rather a questionable approach. To perform this kind of analysis, the 2 populations should be homogenous and similar for the main parameters investigated. Conversely, the Staton et al do not explain how they tested the homogeneity of the 2 populations, particularly with regard to genetic background and prevalence of the traditional cardiovascular risk factors. Too many differences (first of all, age) between such distant and different populations are detectable to correctly perform this analysis.

In fact, the analysis performed in their single population demonstrated no significant relationship between both the polymorphisms and the ischemic stroke; the only significant result derives from pooling data from the study by Lichy et al[5] and is also determined by an overall low number of homozygotes for the AA genotype (n=32), thereby not reaching a sufficient statistical power.

In consideration of all these limitations, we do not believe that the conclusions stated by Staton et al, ie, high levels of protein Z prothrombotic and 79A allele protective for the ischemic stroke, can be drawn from this study. Because low protein Z plasma levels have been reported in several studies to be associated with different prothrombotic conditions, it can be hypothesized that protein Z is either simply a marker of the disease or influenced by the acute phase, but it is at least questionable, to date, to postulate that high levels of protein Z are associated with prothrombotic conditions and that 79AA genotype is protective for the ischemic stroke.

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## Response:

Dr Sofi and colleagues question our conclusion that the consistency of the association between protein Z genotype, elevated blood concentrations of protein Z, and ischemic stroke strengthens the evidence that increased blood concentrations of protein Z concentrations are causally associated with the risk of ischemic stroke.[1]

First, they propose that our conclusion is not supported by pathophysiology, citing methodological bias as an explanation for previous reports of elevated blood concentrations protein Z in stroke patients. However, they provide no explanation of how the use of a normal plasma pool may have biased the results of the study by Kobelt et al.[2] In the study by McQuillan et al,[3] removal of control subjects with a previous vascular event from the analysis does not change the results or conclusions.

Second, as indicated by Sofi et al, blood concentrations of protein Z in our study were lower than previously reported in several other studies, including their own.[4] The reason for this is unclear, although could potentially be related to prolonged storage of blood samples before measuring protein Z levels.[3] However, irrespective of the cause, our within-study comparisons of protein Z concentrations in stroke cases and controls remain valid because the duration of storage of samples for cases and controls was similar and all laboratory samples were collected, processed, stored, and analyzed in the same manner.

Third, Sofi and colleagues question our approach of pooling results from case control studies to clarify the association between protein Z polymorphisms and ischemic stroke. However, this is a well-established technique that is commonly used for aggregating previous research when individual studies have

insufficient power to detect an association.[5] Taken together with the data by Lichy et al,[6] we believe that our study results are consistent with the conclusion that elevated blood concentrations of protein Z are prothrombotic.

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## Cerebral Venous Sinus Thrombosis: Prevention of Recurrent Thromboembolism

*To the Editor:*

Patients who have recovered from cerebral venous sinus thrombosis (CVST) are at risk for sustaining a recurrence of CVST or other thrombotic events (Table). For example, in the recent International Study on Cerebral Vein and Dural Sinus Thrombosis (ISCVT) that was performed in 624 patients with cerebral vein or dural sinus thrombosis, the cumulative risk for recurrent CVST or other thrombotic events after CVST was 6.5%.[1] Recurrent thrombosis is potentially fatal. Therefore, an important clinical question is whether recurrence of CVST or other thrombotic events can be prevented in this group.

Currently, the standard care for patients with a first episode of CVST is anticoagulation therapy, which is usually prescribed for 3 to 6 months in the absence of an identifiable cause.[2] However, controlled data about the benefit and optimal duration of oral anticoagulation in patients with CVST are missing.[3] More prolonged treatment may be required, because the cumulative risk for recurrent CVST or other thrombotic events after 3 to 6 months of oral anticoagulation therapy ranges between 5.5 and 26% (calculated over a mean follow-up period of 18 to 77.8 months).[2,4,5] At the time of these recurrences, which mostly occurred within 1 year, none of the patients received anticoagulation therapy. In the recent ISCVT study, 58.8% of the patients with a recurrence of thrombotic event were not undergoing anticoagulation therapy.[1] The significance of these observations is underscored by the fact that recurrence of cerebral venous thrombosis after stopping anticoagulation therapy can occasionally run a fatal course.[6]

The need for more chronic treatment is further supported by recent observations on the effects of prolonged anticoagulant therapy in patients with idiopathic venous thromboembolism (either pulmonary or deep venous thrombosis).[7] This study evaluated the efficacy of long-term, low-intensity warfarin therapy (international normalized ratio, 1.5 to 2.0) in preventing recurrent thrombotic events among patients with idiopathic venous thromboembolism who had completed at least 3 months of therapy with conventional-dose warfarin. Compared with placebo, long-term and low-intensity warfarin afforded a risk reduction for recurrent thromboembolism of 64% over a mean follow-up period of 2.1 years.

The practical implication was that clinical practice should presumably consist of this prolonged, low-intensity anticoagulant regime, at least for patients with a prior event of pulmonary or deep venous thrombosis.[7] In this particular study, patients with CVST were not specifically studied. However, it seems justified to extrapolate the findings on pulmonary embolism or deep venous thrombosis to CVST, because the underlying risk factors associated with these conditions are identical.[8] Thus, applying this potential risk reduction of 64% to the ISCVT study, long-term anticoagulation therapy could have prevented recurrent thromboembolism in 15 of the 24 patients where recurrence occurred in the absence of anticoagulants. In other words, prolonged anticoagulation in the ISCVT study could have resulted in a cumulative risk for recurrent CVST and other thrombotic events of 4.1%, instead of 6.5%.

We, therefore, suggest that, to reduce recurrence of thrombotic events after CVST, it may be prudent to treat these patients with long-term anticoagulation therapy. Future research should determine the optimal duration and intensity of oral anticoagulation therapy that is necessary to optimally reduce the risk for recurrence of thrombotic events.

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**Cumulative Risk for Recurrence of Thrombotic Events After a First Episode of CVST (Overview of Published Studies)**

| Study | No. of Patients | Mean Follow-Up (Months) | Duration of OAC Treatment (Months) | Cumulative Recurrence of Thromboembolism (%) |
|---|---|---|---|---|
| ISCVT study[1] | 624 | 18 | Mean of 7.7 | 6.5 |
| Preter et al[4] | 77 | 78 | 3 to 4 | 26.0 |
| Breteau et al[2] | 55 | 36 | 6 (56.4% of patients) | 5.5* |
| | | | 6–36 (12.7% of patients) | |
| | | | >36 (30.9% of patients) | |
| VENOPORT study[7] | Not reported | 22 | Not reported | 5.0 |

*Two patients with protein S deficiency. OAC indicates oral anticoagulation.

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## Inosine, Calcium Channels, and Neuroprotection Against Ischemic Brain Injury

*To the Editor:*

We read with great interest the article by Shen et al,[1] dealing with neuroprotective effects of inosine against ischemic brain injury. The results of their study demonstrated that intracerebroventricular administration of inosine before middle cerebral artery occlusion in rats resulted in a higher level of locomotor activity and less cerebral infarction. In addition, they indicated that coadministration of selective A3 receptor antagonist MRS1191 significantly attenuated inosine-mediated protection. In the electrophysiological study, it was shown that inosine antagonized glutamate-induced excitation in cerebral cortical neurons. The authors proposed that inosine may inhibit glutamate postsynaptic responses and reduce cerebral infarction via the activation of A3 receptors.

Several studies have shown the mechanisms for glutamate-induced excitation of neural cells. In a study we presented earlier, changes in acetylcholine (ACh) release evoked by glutamate was investigated in rat central nervous system.[2,3] In an in vitro study, we showed that glutamate increased the release of ACh from rat striatum, which was inhibited by the *N*-methyl-D-aspartate type of glutamate receptor antagonist MK-801.[3] Furthermore, it was demonstrated that the calcium-channel blocker verapamil significantly reduced glutamate-evoked ACh release and that the inhibitory effect was more pronounced in the presence of magnesium.[2] It might be possible that the calcium-channel blocker could inhibit the *N*-methyl-D-aspartate receptor–dependent calcium current in neural cells. It was also shown that the calcium-channel blocker nilvadipine blocked glutamate-evoked intracellular calcium increase with a concomitant reduction in glutamate-induced apoptosis in purified retinal ganglion cells,[4] suggesting that the calcium-channel blocker might inhibit glutamate-induced apoptotic cell death by blocking calcium influx. Therefore, we would like to know whether inosine and calcium-channel blockers might have a synergistic effect in neural protection in the present study by Shen et al. Although the authors demonstrated that inosine did not alter basal glutamate release, nor did it reduce ischemia-evoked glutamate overflow in the central nervous system, it can be speculated that inosine might interfere with glutamate receptor–mediated calcium conductance in neural cells and might have therapeutic potential as a neuroprotective agent against stroke-induced damage.

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*Response*:

We thank Dr Tsuda for expressing interest in our article.[1] As indicated by Dr Tsuda, increasing evidence has suggested that calcium channels are involved in glutamate-mediated neurodegeneration. In our study, we reported that inosine suppresses glutamate-evoked neuronal excitation in cerebral cortex. It is possible that inosine may have indirect actions on calcium channels through the inhibition of glutamate response during cerebral ischemia. Currently, there are no reports suggesting that calcium-channel blockers have synergistic effects on inosine-mediated response in the central nervous system. Our preliminary studies showed that an N-type calcium channel blocker had a trend toward increasing inosine-mediated protection against ischemic brain injury in adult Sprague-Dawley rats. Animals were injected intracerebroventricularly, before middle cerebral artery occlusion (MCAo), with $\omega$-conotoxin GVIA (0.1 $\mu$g/10 $\mu$L, n=6) or $\omega$-conotoxin GVIA+inosine (25 nmol, n=7). Compared with our previous data,[1] $\omega$-conotoxin GVIA alone did not significantly reduce the size of infarction. Coadministration of $\omega$-conotoxin GVIA and inosine significantly reduced volume of infarction compared with the stroke animals pretreated with vehicle ($52.1\pm28.9$ mm$^3$ versus $187.1\pm9.0$ mm$^3$; $P<0.05$; 1-way ANOVA). Although the mean of infarction volume is further reduced by $\omega$-conotoxin GVIA in the animals pretreated with inosine, the difference in protection between $\omega$-conotoxin GVIA+inosine ($52.1\pm28.9$ mm$^3$) and inosine alone ($118.5\pm18.4$ mm$^3$) is not statistically significant. We observed that the majority of animals that received $\omega$-conotoxin GVIA with or without inosine developed seizures after MCAo. Other studies have shown nifedipine or felodipine suppressed hypoxia-evoked inosine release in rat cerebral cortex.[2] The combined effects of inosine and calcium channel blockers in cerebral ischemia are complicated by such observations. Further studies are needed to definitively address the interaction of inosine and calcium channels.

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## Comment on the *Phosphodiesterase 4D* Replication Study by Bevan et al

*To the Editor:*

We observe with interest the results of the study by Bevan et al,[1] where they attempted to replicate our findings associating the *PDE4D* gene with stroke. We appreciate the effort by the authors and their presentation of their data in some detail. The latter allowed us to compare their results with ours, including some unpublished results. In our original publication,[2] the main findings are summarized in Figure 4, where haplotypes are grouped into 3 categories: the at-risk haplotypes, the "wild-type," and the protective haplotype. Although the definition of the at-risk haplotypes involved multiple markers, the protective haplotype, in our data, is characterized by one single-nucleotide polymorphism, specifically allele A of SNP45. This makes it particularly suitable for a replication in a different population. In Table I of Bevan et al, allele A has frequency of 15.4% in 621 stroke patients and 18.7% in 848 controls. We calculated that this corresponds to 191 copies of allele A and 1051 Gs in stroke patients, and 317 As and 1379 Gs in controls. Applying Fisher exact test gives a 2-sided probability value of 0.02 and a 1-sided probability value of 0.01. For the same allele counts, estimated risk of A relative to G is 0.79, or equivalently risk of G relative to A is 1.26. This agrees well with our estimated relative risk of 1.33 for allele G for all stroke patients (in Table 1 of Gretarsdottir et al[2]). Hence, even though Bevan et al did not consider this result as significant, which could be attributable to the use of somewhat different statistical tests and adjustment for multiple comparisons, we are nonetheless encouraged by these results, especially because we have obtained similar results for SNP45 in a replication study of ours (Gretarsdottir et al, unpublished data, 2005). In summary, we appreciate the difficulties of getting definitive results for a replication study when the effect of the variant may only be modest and proper care has to be taken to adjust for multiple testing of variants and phenotypes. And we agree with the authors that further investigation is needed to develop a better understanding of the role of *PDE4D* in stroke.

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