Switching off Embolization From Symptomatic Carotid Plaque Using S-Nitrosoglutathione

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Background—Current antiplatelet regimens fail to prevent the majority of recurrent strokes. Asymptomatic circulating emboli can be detected by transcranial Doppler ultrasound, are frequent in patients with symptomatic carotid stenosis, and predict recurrent stroke risk. S-Nitrosoglutathione (GSNO) is a nitric oxide donor that appears to have relative platelet specificity. We evaluated its effectiveness in reducing embolization in patients with symptomatic carotid stenosis who already were taking aspirin.

Methods and Results—Twenty patients with ≥50% internal carotid artery stenosis and with ≥3 embolic signals detected during a half-hour screening recording were recruited. All had taken aspirin for at least 7 days. They were randomly assigned in a double-blind fashion to either GSNO (4.4 mmol/kg per minute) or saline placebo for 90 minutes. Transcranial Doppler recordings were made from the ipsilateral middle cerebral artery for 1 hour before treatment and at 0 to 3, 6, and 24 hours after treatment. Before treatment, the mean (range) of embolic signals per hour was 6.9 (3 to 13) in the GSNO group and 7.3 (4 to 12) in the placebo group (P=0.68). GSNO resulted in a rapid reduction in the frequency of embolic signals of 84% at 0 to 3 hours, 95% at 6 hours, and 100% at 24 hours (P<0.0001, P=0.003, and P<0.0001 versus placebo, respectively).

Conclusions—Continued embolization is common in patients with carotid stenosis despite aspirin therapy. GSNO was highly effective in rapidly reducing the frequency of embolic signals in this patient group. Despite its short administration time and its short half-life, it resulted in therapeutic effects lasting 24 hours. (Circulation. 2002;105:1480-1484.)

Key Words: ultrasonics • drugs • platelets • carotid arteries • nitric oxide

Symptomatic carotid stenosis is associated with a markedly increased risk of stroke on the order of 15% in the first year. In contrast, asymptomatic carotid stenosis of the same degree is associated with a much lower risk of only 2% per year. The mechanisms by which the asymptomatic plaque becomes symptomatic are incompletely understood, but plaque surface irregularity and ulceration resulting in the adherence of platelets and subsequent thromboembolism appears to play an important role. The role of embolization is supported by recent studies demonstrating that symptomatic cerebral microemboli (embolic signals) are frequent in patients with symptomatic carotid stenosis but less common in patients with asymptomatic carotid stenosis. That these embolic signals have clinical significance is supported by the finding that they are more common in symptomatic patients with recent events, with increasing degrees of stenosis, and in the presence of plaque ulceration, all of which are associated with an increased stroke risk. Furthermore, the presence of embolic signals in the ipsilateral middle cerebral artery independently predicts future stroke and risk of transient ischemic attack in patients with symptomatic carotid stenosis.

The current treatment of choice for symptomatic carotid stenosis ≥70% is carotid endarterectomy. In patients with 50% to 70% stenosis there is still an increased stroke risk, but the benefits of operation are unclear. Such patients are conventionally treated with aspirin, but this does not abolish the stroke risk. More effective antiplatelet regimens are required both for treating patients with tight symptomatic stenosis before endarterectomy and for patients with <70% stenosis.

Nitric oxide plays an important role in preventing platelet aggregation and adhesion. Conventional nitric oxide donors such as glyceryl trinitrite reduce platelet aggregation but are associated with hypotension. This may be an unwanted side effect in patients with tight, hemodynamically significant carotid stenosis. S-Nitrosoglutathione (GSNO) is an S-nitrosothiol from which nitric oxide (NO) is released by the action of enzymes associated with platelet membranes. In animals and in humans, GSNO has significant antiplatelet action at doses that cause less hemodynamic effect than conventional NO donors. Platelet activation can be prevented by administration of GSNO after coronary angioplasty, and GSNO has been shown to inhibit platelet...
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activation in the setting of acute myocardial infarction and unstable angina.\textsuperscript{13} GSNO has been shown to reduce the rate of asymptomatic embolization after carotid endarterectomy detected by transcranial Doppler ultrasound.\textsuperscript{14,15} During endarterectomy, complete endothelial denudation of a large portion of the arterial wall occurs, resulting in an inability of the endothelium to locally secrete NO, but the vessel wall is otherwise relatively normal. Because of this extensive loss of endothelium, one would expect this to be a situation in which NO donors are particularly effective. Whether they are also effective in the much larger group of patients with acute stroke remains unknown.

In this study, we examined the hypothesis that GSNO can reduce embolization in patients with carotid stenosis who are already taking aspirin. The presence of asymptomatic embolization was determined with the use of transcranial Doppler ultrasound. Using this technique, we identified a group of patients with active embolization in whom to test this therapy.

**Methods**

Twenty patients with active embolization with \( \geq 50\% \) symptomatic internal carotid artery stenosis were recruited. Sixty-five patients with ipsilateral symptoms (stroke, transient ischemic attack, or amaurosis fugax) were screened with a transcranial Doppler ultrasound recording from the ipsilateral middle cerebral artery. Patients having \( \geq 3 \) embolic signals during the 30-minute recording were recruited. Internal carotid artery stenosis was determined by color-coded carotid duplex ultrasound on the basis of recognized criteria, including a peak flow velocity of \( \geq 140 \) cm/s. All patients were pretreated with aspirin (75 to 300 mg/d) for \( \geq 1 \) week before recruitment. Patients with active embolization were randomly assigned to treatment with either GSNO (n=10) or placebo (normal saline, n=10) on a double-blind basis. The study medication was administered as an intravenous infusion for 90 minutes. GSNO was infused at a rate of 2.2 mmol/kg per minute and if tolerated was increased to a rate of 4.4 mmol/kg per minute after 10 minutes. The local research ethics committee approved the study protocol, and all subjects gave written informed consent.

Transcranial Doppler recordings were made with the use of a commercially available system (TC2020, EME/Nicolet Ltd) equipped with a 2-MHz transducer. Recordings were made from the ipsilateral middle cerebral artery through the transtemporal window and the transducer fixed in position by a standard headset. A sample volume of 5 mm, a sweep speed of 5 seconds, and a mean (SD) recording depth of 52.9 (2.6) mm were used. After randomization and before treatment with the study drug, a 60-minute recording was made. After initiation of the study drug, a recording was performed for 3 hours starting at the same time as study drug and then for 1-hour duration at both 6 and 24 hours after initiation of treatment. Recordings of the raw Doppler signal were made onto digital audio tape for subsequent off-line analysis. All analysis was performed blinded to study group and time of recording, with the exception of the initial 30-minute screening, which was monitored on-line by an experienced observer to identify patients with active embolization.

For analysis of all recordings, with the exception of the screening recording, the Doppler signal was played from the digital audio tape into the same transcranial Doppler equipment for spectral analysis; a 128-point fast Fourier transform was used with a time-window overlap of \( \geq 66\% \). Embolic signals were identified by their characteristic visual appearance and chirping sound by means of recent consensus criteria.\textsuperscript{16} An intensity threshold of \( \geq 7 \) dB was used because this has been shown to increase interobserver agreement without excessive loss of sensitivity.\textsuperscript{17} Intensity was determined from the color-coded intensity scale on the spectral display. The peak intensity of the embolic signals and the intensity of the background spectra at the same frequency and part of the cardiac cycle, from the preceding or subsequent cardiac cycle, were determined. Interobserver reproducibility in identifying embolic signals was assessed by 2 observers independently analyzing a separate recording that had been prepared from recordings of the middle cerebral artery from 6 patients with carotid stenosis. Agreement was calculated with the use of the proportion of specific agreement, which estimates the probability that one observer will identify a specific embolic signal if another observer has identified it.\textsuperscript{17} A probability of 1 indicates complete agreement. Observer 1 detected 90 embolic signals, and the agreement of observer 1 with observer 2 was 0.92. Observer 2 detected 89 embolic signals, and the agreement of observer 1 was 0.90.

Comparisons between groups were made by use of the Student’s t test for normally distributed data including the intensity of embolic signals. However, the number of embolic signals was not normally distributed, and therefore differences between groups were determined by the Mann-Whitney U test for nonparametric data. Differences between proportions, such as smoking status, were determined by use of the chi-square statistic.

**Results**

There was no difference between the two treatment arms in mean (SD) age (GSNO, 69.7 [6.8] years; placebo, 69.2 [9.3] years; \( P=0.89 \)), mean (SD) degree of internal carotid artery stenosis (GSNO, 89% [10%]; placebo, 82% [11%]; \( P=0.19 \)), or mean (range) time since last symptoms (GSNO, 23.2 [2 to 65] days; placebo, 22.5 [9 to 67] days; \( P=0.94 \)). There was no difference in the proportion of individuals who were male, current smokers, hypertensive, or diabetic between the two groups. There was no difference in mean (range) embolic signals per hour in the two groups during the pretreatment recording (GSNO, 6.9 [3 to 13]; placebo, 7.3 [4 to 12]; \( P=0.68 \)). All patients tolerated the full dose of GSNO administered with no clinically apparent side effects and no hypotensive episodes, defined as a fall in mean arterial pressure of \( >10 \) mm Hg. No hemorrhagic side effects were observed.

GSNO resulted in a rapid reduction in the frequency of embolization, as shown in Figure, with a reduction of 84% at 0 to 3 hours (\( P<0.0001 \) versus placebo), 95% at 6 hours (\( P=0.003 \)), and 100% at 24 hours (\( P<0.0001 \)). A significant reduction was seen within the first hour of administration, but this increased over the first 3 hours. In the GSNO group, the median (range) of embolic signals per hour was 6.9 (3 to 13) before treatment and 1.7 (0 to 4), 1.0 (0 to 2), and 0.6 (0 to 2) in the first 3 hours, respectively, after the initiation of treatment. The frequency of embolization in the placebo-treated patients did not change significantly over time (Figure). Mean (SD) embolic signal intensity was lower in the GSNO group compared with placebo (13.07 [3.15] versus 16.69 [3.58]; \( P<0.001 \)).

All study subjects were followed up from the time of the first recording. Follow-up was continued until an ipsilateral carotid territory ischemic event (stroke, transient ischemic attack, or amaurosis fugax), death, carotid endarterectomy or angioplasty, or study completion. Mean (range) follow-up period was 31.5 (7 to 87) days in the GSNO group and it was 14.7 (4 to 33) days in the placebo group (\( P=0.05 \)). In the placebo group, 3 patients had recurrent ipsilateral ischemic events (2 transient ischemic attacks, 1 amaurosis fugax). None of the patients in the GSNO group had recurrent events.

**Discussion**

In this randomized, double-blind, placebo-controlled study, the intravenous administration of GSNO resulted in a marked
reduction in asymptomatic emboli signals in patients with actively embolizing symptomatic carotid stenosis. Embolization was almost completely abolished by 6 hours after treatment, and this reduction persisted at 24 hours despite the fact that GSNO was only administered for 90 minutes. In the placebo-treated patients, the pattern of embolization was unaltered over the 24-hour period.

Our results have a number of important implications. First, continued embolization is common despite administration of aspirin. Second, in patients with active embolization, the use of novel antiplatelet agents can lead to a marked reduction in the rate of embolization. Third, the S-nitrosothiols may have clinical use in prevention of thromboembolism in patients at risk of stroke; this now needs evaluation in studies with clinical end points.

Our study was not powered to determine the effect of therapy on clinical events, but there were no recurrent ipsilateral ischemic events in the GSNO-treated group compared with 3 in the placebo group. The follow-up period was short because all suitable patients were referred for carotid endarterectomy.

Considerable evidence suggests that GSNO has relative platelet specificity compared with other NO donors. We have previously shown that it resulted in a marked reduction in asymptomatic embolization after carotid endarterectomy. At the endarterectomy operation site the plaque is removed, leaving an area of internal carotid artery denuded of endothelium. This is a potent surface for platelet adherence. The situation in the symptomatic carotid plaque is much more complex and therefore the results from the post–carotid endarterectomy study cannot be directly extrapolated. The precise mechanisms resulting in thromboembolism from the symptomatic carotid plaque are not fully understood. Plaque ulceration appears to be important and is frequently detected when assessed angiographically or histologically, but it is not always present. The presence of thrombus, determined histologically, is strongly associated with symptomatic status. Acute treatment with antiplatelet agents such as GSNO might be expected to be less effective in this situation in which there is established thrombus, compared with the post–carotid endarterectomy setting, in which small recent platelet aggregates have formed. It is also possible that GSNO may only be active in the presence of large areas of denuded endothelium, which are unable to endogenously produce NO, such as those occurring after endarterectomy. However, our current data demonstrate that GSNO has an effect on embolization from the carotid plaque similar to that after carotid endarterectomy. This extends its potential use from the relatively limited application after carotid endarterectomy to the much larger group of patients with symptomatic carotid stenosis. GSNO must be given intravenously, which limits its clinical use, but newer S-nitrosothiols, which can be given orally, are being developed.

Despite GSNO being given for only 90 minutes, embolization remained abolished at 24 hours. This unexpected finding has a number of possible explanations. The half-life of GSNO is short, but it is possible that it could be bound to albumin (nitrosoalbumin) and other high-molecular-weight thiols and subsequently could be slowly released. Alternatively, this marked reduction in platelet aggregation and adhesion may result in a fundamental change in the carotid plaque. If this is the case, one might expect the effect to be independent of the class of antiplatelet agents. GSNO may have had an effect on carotid plaque that is independent of its
action on platelets and is long lasting. We are examining these alternative hypotheses in further studies. Evaluating the duration of effect through the use of Doppler ultrasound detection of asymptomatic embolization is more difficult outside the acute setting. Although our study demonstrates that the presence of embolization is relatively constant over a 24-hour period in the placebo-treated group, a previous study has demonstrated that if recordings are repeated on later days there is greater variability.

Transcranial Doppler ultrasound has been shown to be highly sensitive and specific for the detection of platelet and thrombus emboli in both in vitro and in vivo models.\(^{21-23}\) Emboli result in short-duration, high-intensity signals caused by increased scattering and reflection of ultrasound compared with the surrounding blood. As covered in the introduction, in patients with carotid stenosis, a large number of studies suggest that asymptomatic embolic signals have clinical significance.\(^{6-10}\) Prospective studies in patients with symptomatic carotid stenosis\(^{8-10}\) and in patients with asymptomatic carotid stenosis\(^{24}\) have found embolic signals to be an independent predictor of combined stroke and transient ischemic attack risk. Their high frequency compared with the clinical end points of stroke and transient ischemic attack, combined with their clinical significance, suggest that they may be useful surrogate markers for the evaluation of new antithromboembolic agents. Our study demonstrates that results can be obtained from studies in relatively small numbers of patients with the use this technique. It may allow the optimal combinations of such drugs to be evaluated in relatively small studies before the testing of their efficacy in much larger clinical trials.

We did not perform studies of platelet aggregation or activation in this study. GSNO has shown to be a potent inhibitor of platelet aggregation through the use of ex vivo samples from normal individuals\(^{25}\) and the normal forearm.\(^{11}\) In pathological states in humans, relevant changes are best assessed distal to the potential site of platelet activation, and samples drawn from the venous circulation may provide limited information. We have shown that GSNO is a potent inhibitor of platelet activation, as determined by platelet surface expression of P-selectin and glycoprotein IIb/IIa, in patients undergoing coronary angioplasty who were already pretreated with aspirin.\(^{12}\) In this setting, we sampled blood from the coronary sinus. In the same study, patients treated with aspirin but who did not receive GSNO showed marked platelet activation after angioplasty in blood sampled from the coronary sinus, whereas less consistent activation was detected with peripheral venous sampling. In patients with carotid stenosis, platelet activation may occur locally and therefore optimal sampling would be distal to the carotid stenosis (ie, in the distal internal carotid artery) or, less optimally, from the jugular vein. For ethical reasons, we were unable to obtain samples from these sites in our patients with carotid stenosis. However, it is likely that such sampling would have revealed effects similar to those after coronary angioplasty and in patients with myocardial infarction and coronary atherosclerosis.\(^{11,12}\)

The reduction in asymptomatic embolic signals seen in this study that used an NO donor suggests that the emboli detected are largely platelet aggregates. Embolic signal intensity was lower in the GSNO-treated group. This is consistent with the platelet aggregates being smaller as well as less frequent in this group. Embolic signal intensity is directly related to embolus size, assuming the embolus composition remains unaltered, although there are technical difficulties associated with interpretation of embolic signal size on the basis of intensity alone.\(^{16}\)

Aspirin fails to adequately prevent embolization in this patient group, but other novel antiplatelet approaches may prove more effective. Studies in the coronary circulation demonstrate an additive effect when the ADP receptor antagonist clopidogrel or the glycoprotein IIb/IIIa antagonists are added to aspirin,\(^{26-28}\) but these results cannot be directly extrapolated to the symptomatic carotid plaque because of important differences in the pathogenic processes, with thromboembolism playing the predominant role for the carotid plaque. There are no similar completed studies in patients with stroke or carotid stenosis. Transcranial Doppler embolic signal detection with a study design similar to our current study could be used to compare the different antiplatelet regimens and identify optimal regimens for testing in large trials with stroke as the end point. The effectiveness of clopidogrel in addition to aspirin in patients with symptomatic carotid stenosis is currently being evaluated, with transcranial Doppler ultrasound microembolic signals used as the end point, in the ongoing Clopidogrel and Aspirin in the Reduction of Embolism from Symptomatic Stenosis study (CARESS study).

This study demonstrates that actively embolizing carotid plaques can be identified by means of transcranial Doppler ultrasound and that in this group of patients, GSNO has a highly potent effect in reducing asymptomatic embolization, even in patients already receiving aspirin. Drug administration for a short time results in a reduction in embolization lasting up to 24 hours.

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References


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