S-Nitrosogluthathione Reduces the Rate of Embolization in Humans

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Background—Antiplatelet agents presently used in the secondary prevention of cardiovascular disease fail to prevent the majority of cases of recurrent stroke and systemic embolization. An evaluation of the efficacy of new agents is hampered by a lack of in vivo models in humans. Asymptomatic cerebral embolic signals (ES) may be detected with the use of transcranial Doppler ultrasonography. These signals are particularly common after carotid endarterectomy, and this provides a situation in which new antiplatelet agents can be evaluated. With this model, we determined the effectiveness of S-nitrosogluthathione (GSNO), a nitric oxide donor with relative platelet specificity, in reducing cerebral embolization.

Methods and Results—Transcranial Doppler ultrasound recordings from the ipsilateral middle cerebral artery were made after carotid endarterectomy in 12 control patients and 12 patients receiving intravenous GSNO from the induction of anesthesia until 2 hours after skin closure. Recording times were 0.5 to 3.5, 6 to 7, and 24 to 25 hours after skin closure. The Doppler signal was recorded onto tape, and analysis for ES was performed, with the investigators blinded to treatment group. All patients received aspirin 300 mg/d before surgery and 5000 IU of heparin during surgery. The median (range) number of ES detected during the initial 3-hour postoperative recording was markedly reduced in the GSNO group compared with the control group: 7.5 (0 to 61) versus 38.5 (1 to 219) (P = 0.018). This difference persisted until 6 hours after surgery.

Conclusions—Despite the administration of aspirin and heparin, frequent embolization occurred and was markedly reduced after the administration of GSNO. This demonstrates the potential use of platelet-specific nitric oxide donors in the treatment of thromboembolic disease. This model of cerebral embolism may allow determination of the effectiveness of new antiplatelet agents in humans. (Circulation. 1998;98:1372-1375.)

Key Words: ultrasonics • drugs • platelet aggregation inhibitors • endothelium-derived factors

Although aspirin is effective in the secondary prevention of thromboembolic disease, many strokes and systemic embolic events occur despite its use, and there is a need for more effective antiplatelet agents. Potential agents include ticlopidine, clopidogrel, the new generation of glycoprotein IIb/IIIa antagonists, and nitric oxide (NO) donors. Current methods available for the evaluation of such agents are not ideal; ex vivo studies such as platelet aggregation provide an indicator of potential efficacy but may not be completely representative of biological effectiveness in vivo. Presently available animal models are not always representative of the situation in humans. Because of the low incidence of outcome events, large, expensive, multi-center clinical trials are required in which as many as 19 000 patients may need to be recruited. A reliable model in which to evaluate the efficacy of new agents in vivo in humans, with small patient numbers, would be useful in bridging the gap between laboratory studies and clinical trials.

Recently, it was demonstrated that circulating cerebral emboli can be detected with the use of transcranial Doppler ultrasonography. Emboli appear as high-intensity transient signals with typical acoustic characteristics. This technique has been shown to be highly sensitive and specific in validation studies both in vitro and in animal models. Embolic signals (ES) have been reported in a wide variety of patient groups with potential embolic sources such as carotid artery disease, atrial fibrillation, and cardiac valvular disease. At carotid endarterectomy, endothelial denudation takes place, and the outer layers of the arterial media are exposed, resulting in a potent thrombogenic surface on which platelet adherence and aggregation occur. Asymptomatic ES are frequent after carotid endarterectomy, and recent studies demonstrate that a high frequency of ES during the early postoperative phase correlates with early stroke risk. This situation provides a potential model in which to test the efficacy of new antiplatelet agents. The frequency of ES in this situation may provide sufficient power to allow the evaluation of therapies in relatively small numbers of patients.
In addition to its effects on vascular tone, NO inhibits platelet aggregation by stimulating soluble guanylate cyclase, thereby increasing cGMP, which leads to reduced platelet aggregation and aggregation. Organic nitrates, which act as nitrosothiol from which NO is released by the action of S-nitrosoglutathione (GSNO) is a stable nitrosothiol from which NO is released by the action of enzymes associated with platelet membranes. In animals and humans, GSNO has significant antiplatelet action at doses that cause less hemodynamic effect than conventional NO donors. In a previous study, we demonstrated that platelet activation occurs after coronary angioplasty and that this activation can be prevented by the administration of GSNO. GSNO has also been shown to inhibit platelet activation in the setting of acute myocardial infarction and unstable angina. In the present study, we examined the hypothesis that GSNO prevents platelet aggregation and adherence and therefore subsequent cerebral thromboembolism, as determined with Doppler ultrasound, immediately after carotid endarterectomy.

**Methods**

We studied 24 patients who were undergoing carotid endarterectomy for symptomatic internal carotid artery stenosis of >70% determined angiographically with the European Carotid Surgery Trial method of measurement. Their demographic characteristics are summarized in the Table. The study was approved by the King’s Healthcare Ethical Committee, and informed consent was obtained from all subjects. All patients were pretreated with aspirin 300 mg/d for >1 week before surgery and were administered 5000 IU of sodium heparin intravenously during the operation and before carotid clamping. Patients were allocated either to treatment with GSNO (n = 12) or to no additional treatment (n = 12). In view of the potential side effects of GSNO (eg, hypotension and bleeding), neither the surgeon nor the anesthetist was blinded to treatment, but all data analysis was performed with the investigator blinded to the study group.

GSNO was administered as an intravenous infusion beginning at the induction of anesthesia at a rate of 2.2 nmol · kg⁻¹ · min⁻¹ and, if tolerated, increasing to a rate of 4.4 nmol · kg⁻¹ · min⁻¹ at 10 minutes and continuing until 2 hours after skin closure. The criterion for termination of the infusion was a drop in mean arterial pressure of ≥10 mm Hg, and the infusion was stopped permanently.

A commercially available transcranial Doppler machine (TC 4040; EME/Nicolet Ltd) was used to record from the ipsilateral middle cerebral artery. A sample volume of 5 mm and a mean recording depth of 52.77 mm (range, 48 to 56 mm) were used. Recordings were made to digital audiotape for offline analysis. In all patients, a 1-hour recording was made in the 24 hours preceding surgery. Postoperatively, recordings were made for 3 hours, beginning 30 minutes from skin closure, and for 1 hour at 6 and 24 hours after skin closure.

Analysis of recordings was performed by an observer (J.M.) who was blinded to the clinical details, time of recording, and study group. ES were identified by their characteristic visual appearance and chirping sound. An intensity threshold of >7 dB was used because previous studies have shown that this improves interobserver reproducibility in the detection of ES. The intensity of the ES was calculated by comparison with the built-in intensity scale on the Doppler as previously described. Interobserver reproducibility of ES detection was determined by 2 observers who independently analyzed 4 hours of tape recording from 6 patients with symptomatic carotid artery stenosis. The proportion of specific agreement between observer 1 and observer 2 (H.S.M.) was 0.98 and that between observer 2 and observer 1 was 0.92. For both validation and patient studies, the timing of all ES was noted, and each signal was saved to the hard drive for an analysis of intensity.

The number of ES in each group was not normally distributed, and therefore comparison between the number of ES detected in each group was performed with the Mann-Whitney U test for nonparametric data. For comparison of ES intensity, an unpaired t test was used.

**Results**

Details of the patient groups are given in the Table. All patients underwent technically successful carotid endarterectomy. Of the 12 patients allocated to receive GSNO, 10 tolerated the full dose with no clinically apparent side effects. In 2 patients, there was a fall in MAP of ≥10 mm Hg, and the infusion was suspended. The infusion was tolerated at the half-maximum dose in 1 patient, but in the second patient, even this infusion rate caused hypotension, and GSNO administration was stopped.

In the control group receiving no GSNO, the median (range) number of ES detected during the 3-hour postoperative recording period was 38.5 (1 to 219). At 6 hours, the median (range) number of ES per hour had fallen to 5.5 (0 to 105). By 24 hours, the median (range) number of ES per hour had fallen to 0 (0 to 30).

On an intention-to-treat analysis, there was a significant reduction in asymptomatic embolization in the GSNO group during both the initial 3-hour recording period and hour 6 (Figure 1). The median (range) number of ES detected in the first 3-hour recording was 7.5 (0 to 61) (P = 0.018 versus controls). During hour 6, the median (range) number of ES was 0 (0 to 41) (P = 0.014 versus controls). By 24 hours, the rate of embolization in both groups was low, and there was no difference between control and treatment groups: the median (range) was 0 (0 to 37) (P = 0.74 versus controls).

Individual numbers of ES in the 2 groups during the first 3 hours are given in Figure 2. The 2 patients with frequent ES in the GSNO group were the 2 in whom a full dose of GSNO could not be given because of hypotension. Exclusion of both patients who did not tolerate a full dose increased the difference between the 2 groups: at 3 hours in the GSNO group, the median (range) number of ES detected was 23 (0 to 171) versus 2 (0 to 41) in the control group (P = 0.001 versus controls).
group, the median (range) number of ES was 5.5 (0 to 20) ($P=0.005$ versus controls); at 6 hours, the median (range) was 0 (0 to 7) ($P=0.003$); and at 24 hours, the median (range) was 0 (0 to 3) ($P=0.47$).

A total of 715 ES were detected in the initial 3-hour postoperative recording in the control group compared with 197 in the GSNO group. During this period, ES in the GSNO group were significantly less intense than those in the control group: the mean (SD) was 12.30 (4.30) versus 14.27 (4.71) dB ($P<0.0001$).

In the control group, three patients experienced perioperative ischemic events. One patient had a stroke 20 hours after surgery in the ipsilateral internal carotid artery territory with right facial and arm weakness and dysphasia; this patient recovered fully over a 3-day period, and a CT brain scan showed a cortical infarct. Two additional control patients, both of whom had contralateral carotid occlusion, had strokes in the contralateral internal carotid artery territory. In 1 patient, aphasia and hemiparesis were noted on recovery from anesthesia, and he was left with a residual deficit; a CT scan showed a large area of infarction in the internal carotid artery watershed areas. The second patient developed left hemiparesis and coma 3 days after surgery and died; a brain CT scan showed an intracerebral hemorrhage. There were no strokes or transient ischemia attacks in the GSNO group, but 1 patient was noted to have developed internal carotid artery occlusion on the side of the endarterectomy on repeat carotid duplex before discharge.

**Discussion**

GSNO resulted in a highly significant reduction in the frequency of embolization after carotid endarterectomy. This reduction was maintained at 6 hours after surgery, even though the infusion was stopped 2 hours after surgery. In the majority of patients, GSNO was well tolerated, but in 2 individuals, it resulted in a drop in blood pressure. In 1 patient, it could be continued at half the maximum dose, but in the other, it had to be stopped. Our data provide further evidence for the importance of NO in preventing platelet adhesion and aggregation in vivo and illustrate the potential use of platelet-specific NO donors that may have relatively less hypotensive effect, for a given antiplatelet effect, than conventional NO donors such as nitroglycerin. GSNO has been administered only as an intravenous infusion, but future oral analogs, or other platelet-specific NO donors, may be effective in preventing thromboembolism. Our results also demonstrate that aspirin alone fails to prevent many embolic events.

The patient numbers were too small and the study was not designed to determine whether there was a significant reduction in clinical events. However, there were no clinical ischemic events in the GSNO group. In the control group, there was 1 minor stroke in the ipsilateral internal carotid territory and 2 strokes in the contralateral internal carotid artery territory. The latter occurred in patients with contralateral occlusion; in 1 patient, the pathogenesis was probably intraoperative hemodynamic ischemia, whereas in the other, it was hemorrhage, probably due to a hyperperfusion syndrome. Nevertheless, there certainly was no increase of events in the GSNO-treated group.

In addition to the lower frequency of ES in the GSNO-treated group, the mean intensity of the individual ES was lower in the GSNO group. Theoretically, ES intensity would be expected to increase with increasing embolic size, and this has been confirmed in experimental models. ES intensity also depends on embolus composition, with thrombi resulting in more intense ES than platelet aggregates in experimental models. Assuming the embolus compositions were similar in the GSNO-treated and untreated groups, our results are consistent with emboli in the GSNO-treated group representing smaller platelet aggregates. However, there are a number of technical difficulties associated with the interpretation of ES composition or size on the basis of intensity alone.

The present study demonstrates that ultrasonic ES detection allows the in vivo efficacy of antiplatelet agents to be evaluated in relatively small numbers of patients. In this study, we tested the ability of GSNO to reduce embolization from an arterial luminal surface surgically denuded of endothelium. However, the same technology may allow the effectiveness of agents to be tested on embolism resulting from other clinical situations, such as percutaneous transluminal coronary angioplasty. The use of this model may allow...
effective initial evaluation of new antiplatelet therapies in small studies before their assessment in large and expensive clinical trials. It should also allow dose-response studies to be performed before such trials are begun.

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**References**

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