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GABA-ergic but not Anti-Cholinergic Agents Re-induce Clinical Deficits after Stroke

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Abstract

Our goal was to determine whether the excitatory (i.e., GABA) neurotransmitter system was important in human stroke recovery. We hypothesized that giving midazolam, a GABA_A agonist, to patients would re-induce clinical deficits to a greater extent than the anti-cholinergic scopolamine. Twelve patients (7M) who had recovered from hemiparesis and/or aphasia after first-time stroke and 10 age-matched, healthy controls underwent double-blinded drug challenge with midazolam and 90-days later with scopolamine, or vice versa. Language was scored for comprehension, naming and repetition, and motor function was tested with the 9-Hole Peg Test (9HPT) in each hand. The drugs were administered intravenously in small aliquots until mild awake sedation was achieved. The primary outcome was the change scores from baseline to the two drug conditions, with higher scores denoting greater loss of function. Ten of the 12 patients had recovered from hemiparesis and 7 from aphasia. The median time from stroke to participation was 9.3 months (range = 0.3 – 77.9 months). For motor function, analysis of variance showed that change scores on the 9HPT were significantly greater in patients using the previously paretic hand during the drug state with midazolam ($p = .001$). Similarly, language change scores were significantly greater among recovered aphasics during the midazolam challenge ($p = .01$). In our study, patients demonstrated transient re-emergence of former stroke deficits during midazolam but not scopolamine. These data provide beginning clinical evidence for the specificity of GABA-sensitive pathways for stroke recovery.

Keywords

Stroke Recovery; GABA; Midazolam; Scopolamine; Sedation

Introduction

Most patients who have suffered a stroke demonstrate some degree of recovery,(1) even as soon as 24-hours after the stroke onset (2,3). Both the contralesional hemisphere and preserved regions on the ipsilesional side have been implicated in the restoration of motor (4) and language (5) function, based on findings with functional imaging(6,7), transcranial magnetic

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stimulation (8) and magnetoencephalography(9). Despite description of alternative brain regions that may come to govern function after ischemic injury, however, the role of neurotransmitters in human post-stroke recovery is less well understood.

Animal studies have sought to establish the direct roles of neurotransmitters after post-ischemic injury, but comparable effects in humans after stroke have been only inferred based on investigational trials with drug treatment without understanding which transmitter systems may be important. Early animal studies by Feeney and his colleagues showed that a single dose of d-amphetamine given to rats 24 hours after surgical resection of the motor cortex produced acceleration of recovery, especially with post-injury behavioral training(10), with subsequent work demonstrating comparable effects after stroke (11,12). There has also been modest success in the use of amphetamines in post-stroke patients (13-16). Although having non-specific effects on heart rate and arousal, amphetamines are hypothesized to have specific effects on central norepinephrine (17). There have also been small trials with varying or transient results using agents with other transmitter mechanisms, including drugs whose actions involve serotonin (18), dopamine (19) or acetylcholine (20).

What it is not known is whether these transmitter systems are necessary for the maintenance of stroke recovery. One possible method to investigate the importance of a given transmitter system to stroke recovery in the recovered state is to show that targeted, transient inhibition of a specific neurotransmitter produces a worsening of recovered function. It has been shown in retrospective chart review that medications such as benzodiazepines, certain antihypertensive agents (e.g., clonidine), dopamine antagonists (e.g., haloperidol), and other agents are associated with poorer motor recovery after stroke (21,22). Based on these observations with benzodiazepines, we showed in case studies that the administration of the GABA_A agonist, midazolam, to recovered stroke and TIA patients transiently re-induced former deficits (23, 24). Since there was open-label administration of midazolam, however, and no other agent was used, we could not address the specificity of a GABA-based effect or control adequately for the non-specific effects of sedation. Moreover, the effects on non-neurologic controls were never established.

The purpose of the present study was to conduct a double-blinded investigation to compare the short-acting GABAergic effects of midazolam with the short-acting anticholinergic scopolamine, utilizing an experimental design in both patients and controls. Although both agents have sedative properties, scopolamine is a belladonna alkaloid whose anticholinergic effects are produced by competitive inhibition of muscarinic receptors (25). Despite the demonstration of practice-dependent motor cortical plasticity in normal humans (26) and memory in animal stroke models (27), there are little data showing both enduring and independent effects to promote recovery by cholinergic agents such as donepezil after human stroke (20,28). Our primary hypothesis, therefore, was that the inhibitory (i.e., GABA) neurotransmitter system is selectively important in stroke recovery; thus midazolam would have a greater impact on the re-emergence of clinical deficits in patients than scopolamine.

Methods

Subjects

Patients were English speakers with first-time clinical stroke who had presented at the time of admission with hemiparesis, aphasia and or left hemi-neglect, and a diffusion-positive MRI of the brain. Each subject had to have demonstrated at least some degree of clinical recovery by history from the time of stroke onset. At the time of study entry, patients with prior aphasia could only have mild residual language deficits. Those with prior hemiparesis could only have mild-moderate deficits in the upper extremities so that they would be capable of manipulating the pieces from our peg-placement task. Exclusions included dementia, psychiatric illness, any

other known neurological condition, complete occlusion or significant ($\geq 80\%$) carotid stenosis, or taking any of the following classes of agents: benzodiazepines, antidepressants, cholinesterase inhibitors, CNS stimulants, antipsychotics, opioids, prescription sleeping aids within the previous 48 hours of drug challenge, MAO inhibitors, or antiparkinsonian agents. Normal controls had to be English-speaking right-handers, at least 45 years old, with no history of stroke and meeting all other exclusion criteria. All participants signed Columbia IRB-approved consent.

Methods

General Experimental Design—After giving informed consent, stroke subjects were assigned to undergo drug challenge either with midazolam or scopolamine by a pre-established randomization sequence known only to the study anesthesiologist. On the day of the challenge, each subject was positioned supine with the head up at 60°. Vital signs were monitored, as previously described, and patients received supplemental oxygen by nasal cannula (23). Subjects underwent baseline examination with the study tests for language (aphasia), visual spatial (hemineglect) and motor (hemiparesis) function (See below). After the examiners left the room, the drug (either midazolam or scopolamine) was administered by the anesthesiologist. Midazolam was given as an IV bolus of 2 mg, titrated in 0.5 or 1 mg aliquots until the subject was unable to count backward without error from 20 to 1; scopolamine was given in a .2mg bolus and then 0.1 or 0.2 mg doses also until counting was impaired and there was a 10 cm change in the vision near point. The maximum doses of midazolam and scopolamine were 5 mg and .5 mg, respectively; to insure safety and because higher amounts of drug result in subjects falling asleep. After mild sedation was achieved, the investigators were then brought into the laboratory and the study tasks were repeated. Both the study subject and the examiners were blinded to medication. After 2 hours from the time sedation began, the battery was given once again to insure return to baseline levels. Ninety days later, the stroke patients returned to the laboratory and underwent the entire challenge paradigm with the other drug so that we could compare both agents in the same set of subjects. Normal controls also underwent two challenge sessions 90 days apart, except that each received the same drug on both occasions so that we could determine whether there was a practice effect within subjects associated with agents administered in the second sessions.

Tasks—Based on our prior case series(23,24), the test battery had to be brief, quantifiable, and representative of the functions typically included in more comprehensive clinical assessments. In this case, the peak effect of midazolam, the shorter acting of the two agents, is only about 4-5 minutes at the doses we administer once our sedation criteria are met, so the assessment of motor, language and visual-spatial function was tailored accordingly.

Motor Function was assessed via the 9-Hole Peg Test (9HPT) (29) in the previously-affected and unaffected upper extremities. The 9HPT requires motor coordination, accurate reaching and sensorimotor integration (30). The test consists of a square board with 9 holes spaced 1.25 in apart in a 3 × 3 array. Each peg is 1.25 in long and 0.25 in diameter. The pegboard was centered in front of the patient with the pegs placed in a container next to the board on the same side as the hand being tested. Patients and normal controls were instructed to pick up the pegs one at a time using the targeted hand and to put them as quickly as possible into the holes in any order until all the holes were filled, and then to remove them and return them to the container. The outcome measure was the time to complete this task for each hand, respectively. In order to reduce variability due to extremes of 9HPT times, performances with times longer than 60 seconds were assigned a value of 60 seconds, representing 7 standard deviations below the mean for normal subjects at the oldest age range in our sample.

Language was evaluated as a composite of comprehension, repetition and naming, using the same behavioral tasks as those described in our prior work with midazolam (23,24) and more recently in aphasia recovery (31). These functions were chosen because they encompass skills assessed in widely-used aphasia batteries.(32) Using the Boston Diagnostic Aphasia Examination(33), we derived the assessment of comprehension from the “Commands” subtest in which there were 15 scorable elements, and the evaluation of repetition with 5 items from “Repeating Sentences and Paragraphs”. Naming was evaluated via administration of the abbreviated Boston Naming Test in which there were 15 items.(34) The maximum composite language score was 35. Dictated material that was part of repetition and comprehension was presented via digitized audiofiles to insure rapid assessment in a standardized administration; pictures appeared on a computer screen.

Visual-spatial tasks consisted of line bisection and letter cancellation measures presented on the computer screen and interspersed among the motor and language tests, as previously described (23). Since no patient in this study had a known history of left or right hemineglect, detailed data will not be presented here.

Data Analysis—For our hypothesis regarding the effects of these agents, the dependent measures were the change scores from the pre-drug (baseline) to the drug state. For motor function, it was the difference in time needed to complete the 9HPT, with longer times denoting worse function. Only patients with known hemiparesis in the past were included in this analysis. For language testing, it was the point difference on the language battery in individuals with prior aphasia before and during drug, with higher scores corresponding to poorer function. To achieve the greatest specificity in the assessment of motor-function change scores, the analysis of interest was an analysis of variance looking at the main effects of drug (midazolam vs scopolamine), hand (affected/right vs unaffected/left), and subject (patient vs control) on change scores, and then for interactions. Similarly, language was analyzed with drug and subject as independent factors, with the language change score as the dependent measure.

Results

Twelve patients (7M) and 10 normal control subjects (4M) participated. The mean age of the stroke patients was 59 yrs (SD=11) with a median of 9.3 months since stroke (range = 0.3 – 77.9 months). The mean age for controls was 58 years (SD=10). Among the stroke patients, 11/12 were right handed as were all the controls; 11/12 had left hemisphere strokes. At the time of their original syndromes among the 12 patients, 5 patients had hemiparesis alone, 2 had aphasia alone, and 5 had aphasia and hemiparesis. Table 1 shows patient demographics, lesion locations and the initial clinical syndromes. None of the patients for whom consent was obtained was later dropped from the study because of an inability to perform study tasks at baseline.

The baseline (pre-drug) motor performance of our control subjects (all right-handed) on the 9HPT matched that of normal-functioning adults. (29) The mean performance at baseline for the right hand in our normal controls, shown in Table 2, was 21.2 sec (SD = 3.69), compared to 22.8 sec (SD = 2.53) in published norms for individuals 45 – 75 years of age. Similarly, the mean performance for the left hand in our controls was 22.8 sec (SD = 4.1), compared to 22.31 sec (SD = 2.20) in the literature. Among the normal controls, all of whom were given the same drug on both assessments, there was no statistical difference between baseline scores in the first test session on the 9HPT and in the second test session 90 days later, or between the change scores associated with the first and second drug administrations. Similarly, the baseline language scores were no different between the first and second test sessions, as were the average language change scores from baseline to drug across the two test sessions. As a result, we averaged the change-score data for the 9HPT for each control subject for both test sessions, as

well as for the language tests. Since there were no statistical differences on t-tests for change scores for the right vs left hands for midazolam ($p = 0.44$) or scopolamine ($p = 0.37$), respectively, in our normal controls, and since 9/10 patients had prior paresis on the right, we compared the change scores in the patients' affected hands to the right-hand scores for normals, and unaffected hands to the left hands for normals.

Although more than one-half of the patient subjects were studied within the initial six months after stroke, there was no improvement in baseline (pre-drug) function over the study period. For patients with a history of hemiparesis, there was no statistical difference between mean baseline scores in the first test session on the 9HPT (29.9 sec) and in the second test session (30.1 sec) 90 days later ($p = 0.90$). Similarly, the baseline language scores among prior aphasics were no different between the first and second test sessions.

The amount of study medication administered to patients and controls did not differ. The mean dose of midazolam given to patients was 3.50 mg (SD = 0.5), compared to 3.56 mg (SD = 0.68) given to controls ($p = 0.83$). The mean dose of scopolamine given to patients was 0.46 mg (SD = 0.11), compared to 0.42 mg (SD = 0.04) given to controls ($p = 0.34$).

As seen in Table 2, there was a main effect of drug in producing slowed performances on the 9HPT across all subjects ($p < 0.001$). Figure 1 displays the change scores on the 9HPT. Midazolam produced significantly greater change scores than scopolamine in patients ($p < 0.001$), but in controls as well ($p < 0.001$). With regard to our hypothesis, however, we found that there was a subject $\times$ hand $\times$ drug interaction ($p = .001$) so that the significant lateralized change in motor function was seen in patients using their affected hand under midazolam. There was no statistical difference in the change score as a result of midazolam between the "unaffected" hand in patients, and either the right or left hand for controls.

Prior to the administration of drug, the mean baseline composite-language performances for patients with prior aphasia was 33.25 (SD = 1.6) compared to 33.47 (SD = 2.1) for controls, which was not statistically different. Language change scores are shown in Figure 2. Under the effects of midazolam, the average loss of language for patients who had suffered aphasia was 4.14 points (SD = 2.8), compared to scopolamine which actually showed a 1.4 point improvement ($p = .004$), as designated by the minus sign on the y-axis in the figure. For the normal controls, the mean language change score under midazolam was 0.7 (SD = 1.10) and under scopolamine was -0.2 (SD = 0.74), which was not significantly different. The drug $\times$ subject interaction showed that the only significant worsening of function occurred in patients under midazolam ($p = 0.01$). Among the four patients who had suffered a stroke with right hemiparesis without aphasia, the average language change score was 0.0 (data not shown) following midazolam administration, indicating that the drug effect was specific to those with a history of aphasia.

No patient or control demonstrated neglect on line bisection or letter cancellation during the administration of midazolam or scopolamine.

Discussion

Our results show that the GABA_A agonist midazolam transiently re-induced former motor and language deficits in patients who had shown stroke recovery, whereas the anti-cholinergic scopolamine produced changes no worse than controls. The double-blinded design with two well-known, anesthetic agents demonstrated that the presence of sedation, per se, was not a determining factor in the outcome. Rather, it was the administration of an agent promoting GABA-type inhibition. These findings provide new support for the notion that GABA-inhibitory mechanisms, presumably in relation to excitatory counterparts such as glutamate, may play an important role in the maintenance of recovered function after ischemic injury.

The experimental design in our study allowed us to establish both the sensitivity and specificity of the effect of GABA on post-stroke recovery by having patients and non-neurologic subjects serve as their own controls. In this manner, we were able to demonstrate that beyond the nonspecific effects of sedation, focal motor deficits emerged only in stroke patients with prior hemiparesis, aphasia re-emerged only in patients with former language impairment, and left hemi-neglect, which was present in no patients at time of stroke, appeared in none of the patients under the GABA condition.

One of the mechanisms by which experience-dependent plasticity is thought to occur involves changes in synaptic strength via long-term potentiation (LTP) and long-term depression (LTD) (27). Explored most frequently in relationship to memory and the CA1 region of the hippocampus (35), there is recent evidence that LTP may play an important role in post-injury recovery in other parts of the brain (27,36), including after stroke (37). Glutamate, the primary excitatory neurotransmitter, seems to play a central role in the synaptic strengthening that occurs with LTP. It is increasingly believed that ischemia, in addition to the devastating consequences of primary and secondary cell death, may also be the triggering mechanisms for plasticity in adjacent neurons via induction of LTP (27,38).

We propose that the administration of midazolam, a GABA_A agonist, affected a recovery system by temporarily disrupting regions responsible for post-stroke recovery. This hypothesis is consistent with work by Hagemann et al, who induced focal cortical infarcts in rat somatosensory cortex in vitro, and showed that LTP induction was facilitated ipsilesionally in slices of infarcted animals 1 week after lesion induction but not in homotopic contralateral areas (39). The mechanism thought to underlie this peri-lesional changes is a decrease in GABA_A receptor sites and an increase in NMDA receptor binding sites (40), thereby enhancing cortical excitability after stroke and facilitating the induction of synaptic plasticity (37).

Shallert and colleagues reported the inhibitory effects of diazepam, another GABA_A-agonist, on sensorimotor recovery following experimental lesions in rats (41). Diazepam administered during the first 3 weeks following the brain lesion prevented any measurable functional recovery. Such findings support the retrospective observation that administration of long-term diazepam to stroke patients appears to inhibit recovery(21). The effects of midazolam were seen in our study as early as 10 days after stroke and as remote as 6 years, suggesting long-term vulnerability of transmitter-based aspects of the stroke recovery mechanism.

The finding in our study that administration of midazolam affected motor function significantly more than scopolamine in both patients and normals is consistent with previous work in animal models, showing the overall importance of GABA in motor pathways. Motor cortex injections of muscimol in feline and primate experiments reliably produce contralateral hemiparesis (42,43), similar to the paresis commonly seen in patients after local, intra-arterial injection of barbiturates during Wada testing (44,45). Bntefisch et al found that administration of lorazepam, another GABA_A agonist, to normal subjects reduced substantially use-dependent plasticity in motor cortex, presumably mediated by LTP (46). The specificity of our findings that midazolam had the greatest effect on the affected hand in patients compared to the unaffected hand, to either hand in controls, and to motor function under scopolamine in both patients and controls supports the impact of inhibitory agents on human post-stroke function.

Based on our prior work in which open-label midazolam was administered alone, it was not possible to rule out that in addition to direct GABA_A – mediated inhibition of compensatory mechanisms, midazolam-induced global hypoperfusion was reducing the ability of peri-lesional brain regions to carry on new function, or whether there are fewer neurons in the compensatory zone which participate in the target function, making them vulnerable to lowered CBF (47). But the absence of a major effect to unmask former deficits by scopolamine largely

set this issue to rest since cholinergic neurons also reduce cerebrovascular flow in both animal (48,49) and human models (50,51).

The criteria for determining when patients were sufficiently sedated by effects of the two study medications were based effects on mental status and a change in vision for scopolamine, and so it is possible that the non-sedating pharmacological impact of midazolam and scopolamine were not comparable based on the doses administered. That there was no difference in doses between patients and controls, however, and the fact that patients demonstrated the greatest loss of motor and language function under midazolam support drug specific effects in our experimental model. Nevertheless, additional studies with non-sedating agents with similar mechanisms of action will be needed to confirm our findings. Our stroke cohort and non-neurological controls were relatively young, and so the effect of these agents on an older stroke population has not yet been determined. We also did not have explicit information regarding the nature and extent of deficits at the time of stroke onset, although the outcomes here were consistent with the hypothesized, selective impact of drugs on recovered function. There needs to be prospective evaluation of patients from the acute to the chronic periods to establish more precisely the relationships among deficits at stroke onset, the degree of stroke recovery and the susceptibility to these transmitter effects. The role of imaging in our experimental model has also yet to be explored. Finally, with a larger cohort, we will also be able to compare the effect of recovery of functions on each other (e.g., language and motor skills) in the same patients.

In summary, we were able to demonstrate in a unique clinical experimental model in human stroke patients that enhancement of an inhibitory neurotransmitter system via administration of a GABA_A agonist was sufficient to disrupt plasticity that supported functional stroke recovery. Future studies will be needed to determine how such mechanisms may be translated into pharmacological therapies that could promote the restitution of function after ischemic injury.

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Figure 1.

Mean motor change scores (sec) for the affected and unaffected hand in 10 patients, and the right and left hand in 10 normal controls. Dark bars denote performances associated with midazolam; light bars denote scores for scopolamine. One standard deviation is shown by the line above each bar. Only patients with a prior history of hemiparesis are shown.

Figure 2.

Mean language change scores (out of maximum of 35 points) for 7 patients with a history of stroke-induced aphasia and for 10 normal controls. One standard deviation is depicted by the line above each bar. Only patients with a prior history of aphasia are shown.

Table 1

Patient characteristics, Time from clinical event to first drug challenge, and clinical state at stroke onset.

<u>Patient</u>	<u>Age,y/Sex</u>	<u>Lesion Site</u>	<u>Months Since Stroke</u>	<u>Initial Clinical Syndrome</u>
1	77/M	L medulla	0.3	R hemiparesis
2	51/F	L basal ganglia	0.7	R hemiparesis
3	45/F	L external capsule	76.8	R hemiparesis, aphasia
4	56/F	L internal capsule/ basal ganglia	76.8	R hemiparesis
5	M/70	L pons	77.9	R hemiparesis
6	45/M	L superior temporal	1.1	Aphasia
7	51/M	R basal ganglia	5.3	L hemiparesis
8	52/M	L temporoparietal	13.3	Aphasia
9	73/M	L insula/corona radiata/basal ganglia	73.7	R hemiparesis, aphasia
10	56/F	L basal ganglia	3.8	R hemiparesis, aphasia
11	61/F	L basal ganglia	52.0	R hemiparesis, aphasia
12	72/M	L frontal operculum/ anterior insula	2.2	R hemiparesis, aphasia

L = Left;
R = Right;

Table 2

Means and (standard deviations) for times (in seconds) to complete the 9Hole Pegtest for the affected and unaffected hands for patients and controls administered before (Baseline) and during (Drug) midazolam and scopolamine.

		<u>Patients</u>		<u>Controls</u>	
		Affected Hand	Unaffected Hand	Right Hand	Left Hand
Midazolam	Baseline	28.29 (7.05)	22.71 (3.83)	21.1 (3.69)	22.8 (4.1)
	Drug	49.00 (13.11)	37.09 (10.55)	31.2 (7.4)	31.4 (5.68)
Scopolamine	Baseline	30.01 (5.55)	23.04 (3.79)	21.1 (3.69)	22.8 (4.1)
	Drug	38.48 (12.55)	29.55 (12.42)	25 (4.74)	27.0 (5.96)