Safety and Tolerability of SonoVue® in Patients with Large Artery Anterior Circulation Acute Stroke

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ABSTRACT

BACKGROUND AND PURPOSE: Ultrasound contrast agents (UCAs) are routinely used to improve the visualization of intracranial arteries. Since a higher rate of intracranial hemorrhage (ICH) has been observed in patients undergoing sonothrombolysis in combination with UCAs, we conducted this study with the aim of assessing safety and tolerability of SonoVue® in patients with acute ischemic stroke due to anterior circulation large artery occlusion (LAO) and eligible to intravenous thrombolysis and/or mechanical thrombectomy.

METHODS: Among 474 patients consecutively admitted to our Stroke Unit with anterior circulation ischemic stroke, SonoVue® was administered during transcranial ultrasound evaluation to 48 patients with suspected LAO for diagnostic confirmation (group I) and to 44 patients with inadequate temporal bone window. Forty-eight stroke patients with LAO diagnosed only by computed tomography (CT) angiography /magnetic resonance (MR) angiography and matched for age, gender, and National Institutes of Health Stroke Scale score with group I represented the control group (group II). Thrombolysis, thrombectomy, or combined treatment were offered to all eligible patients. Brain MR imaging/CT was performed in both groups in case of neurological deterioration or after 1 week to check for ICH.

RESULTS: SonoVue® did not cause any serious adverse event; only mild and transient side effects were reported in six cases (6.5%). Among patients in groups I and II, there were 31 (32.3%) secondary cerebral bleedings with no statistically significant difference between the groups, but only 2 (2.1%) were symptomatic.

CONCLUSIONS: According to our study, SonoVue® can be safely administered to acute ischemic stroke patients with suspected anterior circulation LAO and/or inadequate temporal bone window.

Keywords: Ultrasound contrast agents, acute ischemic stroke, large artery occlusion, thrombolysis, thrombectomy.

Acceptance: Received October 9, 2016. Accepted for publication November 10, 2016.

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Acknowledgments and Disclosure: This study did not receive any academic and/or external funding. The authors have no conflicts of interest to report for this study.

J Neuroimaging 2017;27:409-413.
DOI: 10.1111/jon.12416

Introduction

Five recently published randomized controlled trials (RCTs) demonstrated an amazing superior benefit of combining endovascular mechanical thrombectomy with intravenous thrombolysis over intravenous thrombolysis alone in acute ischemic stroke patients with large artery occlusion (LAO) in the anterior circulation.[1] All these RCTs used noninvasive arterial imaging to select patients with an occlusion of the terminal internal carotid artery (ICA), M1 or M2 segment of the middle cerebral artery (MCA). This might be a reason why such RCTs were positive, in contrast to the previous thrombectomy trials (IMS III, MR RESCUE, SYNTHESIS).[2–4] Accordingly, intracranial vessel occlusion should be diagnosed with noninvasive imaging whenever possible before considering treatment with mechanical thrombectomy.[5]

Transcranial ultrasound is the prototype of noninvasive neurovascular imaging and in acute stroke patients, it can evaluate up to 16 proximal intracranial arterial segments with the goal of detecting normal, stenosed, or occluded intracranial vessels.[6] Additionally, it is the most convenient method to detect collateral flow and the hemodynamic significance of extracranial or intracranial steno-occlusive lesions, monitor recanalization during thrombolytic therapy in real-time, determine stroke pathogenic mechanism, and select the next and most appropriate step in patient management.[7] A fast-track insonation protocol has also been developed for rapid transcranial ultrasound performance and interpretation, in the emergency setting of acute ischemic stroke. The sequence of fast-track insonation steps is chosen according to the clinical localization of the ischemic arterial territory.[8] Recent findings from a multicenter study support the systematic use of transcranial ultrasound also for the identification of symptomatic intracranial atherosclerosis in patients presenting with acute ischemic stroke.[9]

However, transcranial ultrasound may be hampered by insufficient acoustic bone windows, by unfavorable insonation angles, by low flow volumes or low flow velocities in single arterial segments, or by a combination of these conditions. In acute stroke patients, the basal cerebral arteries can only be detected

in 55–80% of cases with unenhanced transcranial color-coded Doppler sonography (TCCS). Intravascular ultrasound contrast agents (UCAs) were originally designed to improve conventional ultrasound imaging.[10,11] Noteworthy, contrast-enhanced TCCS has a number of distinct advantages over CT and MRI: it can be performed immediately, without any preliminary laboratory testing, and it can be carried out at bedside and in real time so that rapid changes can be captured. In case of a suspected intracranial occlusion, UCAs are administered intravenously for confirmatory purposes. In fact, a missing flow signal not necessarily implies occlusion, it could represent hypoplasia/aplasia. It is therefore helpful to use UCAs, in addition to checking for indirect signs in the proximal and distal vessel segments.[12]

Yet, the physical changes of UCA microbubbles induced by ultrasound can cause strong mechanical stress to the adjacent endothelial cells and vascular wall, increasing the vascular permeability to circulating macromolecules. These undesired effects of UCAs might increase the risk of hemorrhagic transformation especially in acute stroke patients with LAO. This has been demonstrated especially in the setting of microbubble-enhanced sonothrombolysis, where patients underwent prolonged (1-2 hours) transcranial ultrasound monitoring with TCCS or TCD.[13,14] Not all studies are in line with these results having shown contradictory findings with different UCAs (Levovist®, Sonovue®) and occlusion sites (MCA, basilar artery [BA]).[15–17]

Since the advent of mechanical thrombectomy, there has been a wider utilization of UCAs as a diagnostic aid to transcranial ultrasound. Moreover, the percentage of stroke patients undergoing thrombectomy whether associated or not with intravenous thrombolysis is expected to increase in the near future. For this reason, we decided to conduct a study with the aim of assessing safety and tolerability of sulfur hexafluoride microbubbles, SonoVue® (Bracco, Milan, Italy), a long-acting UCA, in patients with acute ischemic stroke due to an LAO of the anterior circulation, and eligible to intravenous thrombolysis and/or mechanical thrombectomy.

Methods

General Study Protocol

Patients consecutively admitted for an acute ischemic stroke at the Stroke Unit of the University of Padua School of Medicine were prospectively assessed during a 2-year study period (June 2014-June 2016) adopting standardized diagnostic and therapeutic procedures as recommended by the European Stroke Organization,[5–18] including treatment with intravenous recombinant tissue plasminogen activator (rt-PA) and/or mechanical thrombectomy in eligible patients. All patient details were entered in a computerized database, recording their demographic characteristics, vascular risk factors, and routine blood tests. Patients were diagnosed as having arterial hypertension, if they had a systolic blood pressure ≥ 140 mmHg or diastolic blood pressure ≥ 90 mmHg or if they were or had been on antihypertensive medication at any time before enrollment. Diabetes mellitus was defined as fasting serum glucose ≥7.0 mmol/L (≥126 mg/dL), nonfasting serum glucose ≥11.1 mmol/L (≥200 mg/dL), glycated hemoglobin (HbA1C) ≥ 48 mmol/mol (≥6.5% by the Diabetes Control and Complications Trial), or the use of glucose-lowering drugs before enrollment. Hypercholesterolemia was defined as total cholesterol >6.2 mmol/L (>240 mg/dL) or use of lipid-lowering drugs before enrollment. Any history of angina pectoris, myocardial infarction, coronary treatment (angioplasty/stenting or bypass surgery), peripheral arterial disease, previous transient ischemic attack, and stroke was also recorded. Any cardiac arrhythmia such as atrial fibrillation was diagnosed on the basis of at least one electrocardiogram (ECG) before or during the study period. Smoking habit was defined as current or cessation within the past 5 years. Clinical and functional status was assessed with the National Institutes of Health Stroke Scale (NIHSS) and the modified Rankin Scale, respectively, both on admission and at discharge. Territorial distribution was evaluated in all patients with magnetic resonance imaging (MRI) or repeat computed tomography (CT) and with the Oxfordshire Community Stroke Project classification system.[19] The diagnostic workup included standard blood tests, routine ECG, cervical vessel ultrasound, transcranial ultrasound, transthoracic echocardiography, and 24-hour ECG monitoring, plus coagulation studies, and transesophageal echocardiography if appropriate.

Ultrasound Protocol

All patients presenting with an acute ischemic stroke were evaluated by specialized stroke neurologists experienced in the use of cerebrovascular ultrasound (CB, FV, SF, FF), who performed a fast-track, clinically oriented cervical and intracranial ultrasound assessment aimed at diagnosing an LAO amenable to intravenous thrombolysis and/or mechanical thrombectomy.[8]

All cervical vessels were examined using high-resolution color-coded duplex sonography scanners with a high-frequency (5-10 MHz) linear probe. Examination was started on the affected side, in transverse B-mode planes followed by color-mode sweep from proximal to distal carotid segments. Carotid and vertebral plaques were rapidly recorded and stenoses were graded according to validated criteria.[20,21]

The intracranial arteries were examined with a low-frequency (1-3 MHz) phased-array TCCS probe following a validated protocol.[22] The system settings were adjusted for the analysis of middle high-velocity signals, with an appropriate pulse repetition frequency for arterial vessel detection. Patients were examined in a supine position, first through the transtemporal bone window of the affected side, and then rapidly through the transtemporal window of the contralateral side and the transforaminal bone window. The MCA, the anterior cerebral artery (ACA), the posterior cerebral artery (PCA), and the top of the BA were examined through the transtemporal bone window, while the intracranial segment of the vertebral arteries (VAs) and the proximal-middle BA segments were assessed through the transforaminal bone window. Moreover, the carotid syphon and terminal ICA were examined through the transtemporal bone window, in the anterior coronal plane. After correctly identifying all these vessels, hemodynamic data were collected (blood flow direction, peak systolic velocity [PSV], and end-diastolic velocity [EDV]). The maximum achievable flow velocity without angle correction was recorded, unless a straight vessel segment of at least 1.5-2.0 cm was visualized. The pulsatility index, which reflects downstream intracranial arterial resistance, was calculated for all patients according to the following formula: PSV-EDV/mean flow velocity. Cerebral artery stenosis was diagnosed qualitatively based on aliasing phenomenon visible on TCCS in a short segment of the vessel,

increased flow velocities in the area of the stenosis, and flow disturbances upstream and downstream from the lesion. Previously validated criteria were used to detect ≥50% intracranial stenosis and occlusion.[23] In case of suspected anterior circulation LAO (group I) or unfavorable temporal bone window in eligible patients for reperfusion treatment, 1 cc of SonoVue®, a UCA made up of microbubbles stabilized by phospholipids and containing sulfur hexafluoride, was administered as a bolus via a 23-gauge sterile infusion catheter placed into an arm vein. After injection of the UCA, the catheter was flushed with 5 mL of saline to ensure that all of the preparation had been administered. Symptomatic intracranial arterial stenosis/occlusion was diagnosed when there was evidence of a cerebral infarction on repeat brain CT or MRI in the territory of a ≥50% stenosis/occlusion detected by TCCS and confirmed by enhanced TCCS. For the purpose of this study, group I patients were matched for age, gender, and NIHSS score with a group of patients with anterior circulation acute stroke due to LAO diagnosed by CT angiography (CTA)/MR angiography (MRA) undergoing the same specific treatment who did not receive SonoVue® (group II). In case of clinical deterioration (an increase in NIHSS score of ≥4) or just before hospital discharge, all patients pertaining to both groups underwent brain MRI/CT to check for intracranial hemorrhage (ICH).[24]

Statistical Analyses

The statistical analysis was performed with the SSPS statistical software (SPSS Inc. version 13.0 for Windows, Chicago, IL, USA). Patients' demographic and clinical characteristics are given as means ± standard deviations (SD) or as medians and ranges. Noncontinuous variables are presented as percentages. Frequencies and categorical data were compared with χ^2 or Fisher's exact tests, as appropriate. Significance was assumed at $P < .05$.

Standard Protocol Approval and Patient Consent

The study was approved by our local ethics committee and informed consent was obtained for all patients and control subjects.

Results

During the 2-year study period, a total of 474 patients were hospitalized with an anterior circulation acute ischemic stroke, comprising 280 (59.1%) men and 194 (40.9%) women, with a mean age of 70.3 ± 12.3 years.

On emergency TCCS, 48 (10.1%) patients had a suspected LAO of the anterior circulation: 38 MCA occlusions and 10 terminal ICA occlusions. This diagnosis was confirmed first by enhanced TCCS and then by CTA (46) or MRA (2). Noteworhty, 44 (9.3%) patients had inadequate acoustic bone windows that hampered visualization by TCCS. Therefore, SonoVue® was administered to a total of 92 patients and it was shown to be safe and well tolerated in all these patients, as no serious adverse event occurred soon after UCA administration. Only some mild adverse effects were reported by 6 patients (6.5%): 4 patients (4.3%) complained of a slight pain (1/10, 1/10, 2/10, and 2/10 on the numeric pain rating scale) at the injection site, while 2 patients (2.2%) reported a transient itching around the lips (Table 1).

Table 1. Adverse Events Caused Directly by SonoVue

Adverse event	All patients (92) *n* (%)	48 patients# *n* (%)	44 patients* *n* (%)
Nonserious	6 (6.5)	3 (6.3)	3 (6.8)
- Pain at injection site	4 (4.3)	2 (4.2)	2 (4.5)
- Itching around lips	2 (2.2)	1 (2.1)	1 (2.3)
Total	6 (6.5)	3 (6.3)	3 (6.8)

n = number of patients.
#Patients with large artery occlusion.
*Patients with inadequate acoustic bone window.

Table 2. Demographics, Clinical Characteristics, and Type of Treatment of Patients with Anterior Circulation Large Artery Occlusion (LAO)

Variables, *n* (%)	SonoVue ° (*n* = 48)	No SonoVue ° (*n* = 48)
Age, years (± SD)	69.2 (10.4)	68.8 (11.2)
Men	28 (58.3)	26 (54.2)
Women	20 (41.7)	22 (45.8)
NIHSS at admission (median and range)	18 (10-26)	17 (11-25)
Anterior circulation LAO	48	48
-Terminal ICA	10	10
-MCA	38	38
Hypertension	33 (68.8)	32 (66.7)
Diabetes mellitus	15 (31.3)	14 (29.2)
Hypercholesterolemia	25 (52.1)	24 (50.0)
Smoking	17 (35.4)	15 (31.3)
Cardiovascular diseases	10 (20.8)	10 (20.8)
Atrial fibrillation	14 (29.1)	13 (27.1)
Previous TIA	9 (18.8)	8 (16.7)
History of Stroke	4 (8.3)	4 (8.3)
On antithrombotics	21 (43.7)	22 (45.8)
-Antiplatelets	17 (35.4)	18 (37.5)
-Anticoagulants	4 (8.3)	4 (8.3)
Carotid disease	10 (20.8)	9 (18.8)
Specific treatment	48	48
Thrombolysis (rt-PA)	7	7
Thrombectomy	27	27
Combination	14	14

n = number of patients; BMI = body mass index; CRP = C-reactive protein; NIHSS = National Institutes of Health Stroke Scale; PVD = peripheral vascular disease; SD = standard deviation; TIA = transient ischemic attack; LAO = large artery occlusion; ICA = internal carotid artery; MCA = middle cerebral artery.
° the comparison between these two groups shows no significant difference.

Out of 474 patients with an anterior circulation acute ischemic stroke, 191 (40.3%) arrived to the emergency department within 4.5 hours from symptom onset. However, only 121 patients (25.5%) were treated with intravenous thrombolysis. Among group I patients, 7 received thrombolysis (rt-PA), 27 underwent mechanical thrombectomy, and 14 combined treatment (rt-PA and thrombectomy) with no statistically significant difference emerging between the two groups (Table 2). Overall, there were 31 secondary ICHs (32.2%). Table 3 displays the type of ICHs in the two groups according to the specific stroke treatment. Notably, there were less ICHs in the SonoVue® group (14/31 [45.2%] vs. 17/31 [54.8%]), although the difference was not statistically significant. Only two (2.1%) ICHs were symptomatic: one parenchymal hematoma type 2 in a patient who did not receive SonoVue® and was treated with rt-PA for a distal M1-MCA occlusion; one parenchymal hematoma type 1 remote from the ischemic area in a patient who was administered SonoVue® and who underwent a combination of

Table 3. Posttreatment Intracerebral Bleedings

Type	SonoVue ($n = 48$)	No SonoVue ($n = 48$)
HI 1	4 (1,1,2)	5 (2,1,2)
HI 2	4 (2,1,1)	5 (2,1,2)
PH 1	3 (2,1,0)	4 (2,1,1)
PH 2	0	1 (1*,0,0)
PHr 1	3 (1,1,1*)	2 (2,0,0)
PHr 2	0	0
Total	14	17

n = number of patients; HI 1: small petechiae along the margins of the infarct; HI 2: more confluent petechiae within the infarct area but without space-occupying effect; PH 1: blood clot(s) not exceeding 30% of the infarct area with some mild space-occupying effect; PH 2: blood clots exceeding 30% of the infarct area with significant space occupying effect; PHr 1: small- or medium-sized blood clots located remote from the actual infarct; a mild space occupying effect could be present; PHr 2: large confluent dense blood clots in an area remote from the actual infarct; significant space occupying effect may be present.
In brackets, cerebral bleedings according to specific treatment: rt-PA, thrombectomy, combined.
*Symptomatic intracerebral hemorrhage (sICH): 2/96 = 2.1%.

intravenous thrombolysis and mechanical thrombectomy for an M1-MCA occlusion.

Discussion

The main result of this study is that SonoVue® can be used safely in patients with acute ischemic stroke secondary to an LAO of the anterior circulation and eligible to systemic thrombolysis, mechanical thrombectomy, or both. Only mild and transient adverse effects were reported by a minority of patients receiving SonoVue®, making it a well-tolerated UCA.

UCAs are made up of microbubbles consisting of a gaseous core (air or heavy gas) surrounded by a liquid shell (albumin, galactose, lipids, phospholipids, or polymers)[25] that prevents gas leakage as well as aggregation of microbubbles. SonoVue® in particular is made up of microbubbles stabilized by phospholipids and containing sulfur hexafluoride, an innocuous gas. The high bubble concentration, combined with a favorable size distribution profile, provides SonoVue® with a strong echogenicity.[26] Intravascular UCAs were originally designed to improve conventional ultrasound imaging and SonoVue® was shown to be effective in increasing the detection of normal or pathological flow in the intracerebral arteries: in 66–74%, a nondiagnostic investigation was converted into a diagnostic investigation.[27] In our study, SonoVue® was administered to 48 stroke patients with a suspected LAO of the anterior circulation but also to 44 acute stroke patients with an inadequate temporal bone window. SonoVue® was very well tolerated as no serious events occurred but only mild side effects were described in a small percentage (6.5%) of patients similarly to a series of mainly ambulatory patients.[27]

However, the physical changes of microbubbles induced by ultrasound can cause strong mechanical stress to the adjacent endothelial cells and vascular wall, increasing the vascular permeability to circulating macromolecules. Increase of vascular permeability can be explained by different mechanisms.[28] At high acoustic pressure, inertial cavitation of microbubbles as well as microbubble destruction can transiently (for 20–30 seconds) permeabilize cell membranes (sonoporation) due to shock waves and liquid jets produced by collapse of microbubbles.[29] Cell membrane permeability can be increased by high-intensity ultrasound on its own as well; however, microbubbles significantly increase this effect in the presence of ultrasound with high acoustic pressure.[30] Volumetric changes of oscillating microbubbles (stable cavitation) increase gap-junction distance between endothelial cells simply because of physical expansion of microbubbles leading to distension of vessel wall. Furthermore, mechanical perturbation of cell membranes by microbubble cavitation alters the cell membrane potential and stimulates endocytosis of circulating macromolecules. All these undesired effects of UCAs might explain a higher rate of hemorrhagic transformation reported in two studies on the combined treatment of sonothrombolysis and microbubbles.[13,14] Nonetheless, the higher rate of hemorrhagic transformation might be related to the different type of UCAs used (perflutren-lipid microspheres in the Tucson study instead of common diagnostic galactose-based microbubbles such as SonoVue® used in our study), the different method of administering UCA (continuous infusion instead of bolus), the different type of transcranial ultrasound (TCCS versus TCD), and the different period of exposition to ultrasound (a few minutes as in fast diagnostic protocol vs. 1–2 hours as in sonothrombolysis). In our study, we evaluated the safety of Sonovue® administered as a bolus for diagnostic purposes (confirm/confute an LAO) similarly to a iodinated contrast agent injection during CTA; therefore, exposition to the combined effect of UCA and ultrasound was limited to a few minutes that was the time necessary to reach a diagnosis.

In the routine setting, contrast-enhanced TCCS is accepted as a safe diagnostic method for evaluation of the intracranial arteries in acute stroke.[31,32] Moreover, in an experimental model made up of Wistar rats subjected to filament occlusion of the right MCA, combined contrast-enhanced ultrasound, and rt-PA treatment was shown to be safe.[33] However, the current stroke treatment for patients with an anterior circulation LAO has been recently modified based on the results of five RCTs (MR CLEAN, ESCAPE, REVASCAT, SWIFT PRIME, and EXTEND IA).[5] Therefore, our concern was a potential increased risk of ICH in patients receiving SonoVue® on top of rt-PA and/or thrombectomy. In the above-mentioned trials, parenchymal hematoma type 2 and symptomatic ICH rates were 5.1% and 4.4%, respectively.[34] In our study, the rates of intracerebral bleedings, parenchymal hematoma type 2, and symptomatic ICH in patients receiving SonoVue® were by no means higher than those reported in the trials in which SonoVue® was not administered.[5,35]

In conclusion, this study indicates that SonoVue® can be safely administered in acute ischemic stroke patients with suspected anterior circulation LAO and/or inadequate temporal bone window. Patients receiving SonoVue® and then undergoing a specific stroke treatment (intravenous thrombolysis and/or mechanical thrombectomy) are not exposed to a higher risk of ICH. This study supports the importance of larger clinical trials investigating safety of contrast-enhanced ultrasound in acute cerebral artery occlusion, especially in hospital settings where CTA/MRA is not available or is contraindicated.

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