A Pilot Parallel Double-Blind, Randomized Trial of Galantamine for Subarachnoid Hemorrhage

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Protocol Title:	**A Pilot Parallel Double-Blind, Randomized Trial of Galantamine for Subarachnoid Hemorrhage**
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Study Coordinator:	If a coordinator / research nurse / research assistant has been identified
Population:	A total of 60 subjects who present with aneurysmal subarachnoid hemorrhage, age 18-75 will be enrolled.
Number of Sites:	UT Houston Health Science Center, Memorial Hermann Hospital System and the Methodist Hospital System
Study Duration:	3 years
Subject Duration:	90 days

Protocol Summary

Title: **A pilot, parallel double-blind, randomized trial of Galantamine for Subarachnoid Hemorrhage**

Short title: SAHRANG: SubArachnoid Hemorhage Recovery ANd Galantamine

Population: Patients with aneurysmal subarachnoid hemorrhage in the age group of 18-75 years admitted to the Memorial Hermann Hospital System and the Methodist Hospital System who meet the eligibility criteria for the study will be enrolled.

Number of Sites: Memorial Hermann Hospital System and the Methodist Hospital System

Study Duration: Duration of the study will be 3 years.

Subject Duration: Duration per subject would be 90 days.

Objectives:

Abstract: Subarachnoid hemorrhage (SAH) is associated with significant morbidity and mortality. Galantamine is a medication used in the treatment of dementia. Preclinical studies have shown that galantamine addresses several mechanisms proposed to contribute to worse clinical outcomes after SAH. We propose a pilot safety and tolerability study examining the use of galantamine to improve outcomes after acute SAH. We hypothesize that galantamine started within 36 hours and continued for 90 days will be well tolerated and safe. Our second hypothesis is that galantamine treatment will be associated with a decrease in central and systemic inflammation after SAH.

Aim 1: To investigate the safety and tolerability of galantamine in SAH patients when given 36 hours after admission to hospital and continued for 90 days.

Aim 2: To develop an evidence-based hypothesis for efficacy of galantamine on the 90 day mRS.

Aim 3: To investigate the effect of galantamine treatment on CSF and serum levels of inflammatory cytokines after SAH.

Methods: This is pilot double-blind placebo-controlled randomized trial to evaluate the safety and tolerability of galantamine after SAH. 60 patients will be enrolled. The first 30 subjects will be randomized to either galantamine 8mg twice a day or placebo. The next 30 subjects will be randomized to galantamine 12mg twice a day or placebo. Dose of galantamine will be adjusted as needed if tolerability issues occur. An interim analysis for dose tolerability will be performed after 30 subjects have been enrolled before proceeding to the second phase of the study. CSF and serum samples will be collected at 8 predetermined time points and differences in

inflammatory cytokines between groups will be assessed. Clinical outcomes at 30 days, 60 days and 90 days will be assessed by the modified Rankin Scale, Montreal Cognitive Assessment, EuroQOL.

Conclusion: This is a study to investigate the safety and tolerability of galantamine therapy for the treatment of SAH. In addition we will gain insights into preliminary estimates of efficacy and possible mechanisms of action.

Background Information

Subarachnoid hemorrhage (SAH) is caused by a rupture of a cerebral aneurysm. SAH represents 5-10% of strokes and is a significant public health problem. Mortality is high (up to 40%) and morbidity is significant as patients are often young and are left with cognitive and functional deficits.[1,2] Brain injury as a result of SAH occurs in two stages. The first stage of injury occurs within 72 hours and has been coined early brain injury.[3] The second stage of injury occurs between 4-14 days after rupture and is characterized by cerebral vasospasm (CVSP) and delayed cerebral ischemia (DCI). CVSP and DCI are complications that lead to morbidity and mortality after SAH.[4] Both clinical and animal studies have shown that uncontrolled inflammation is an important contributor to both stages of injury.[5,6] Recent preclinical studies have implicated nicotinic α7 receptors in modulation of inflammation, and have demonstrated that stimulation of these receptors can reduce the levels of inflammatory cytokines.[7]Currently, there is no direct α7 receptor agonist that has been approved for use in humans.

Galantamine is an FDA-approved medication that inhibits acetylcholinesterase and allosterically potentiates α7 receptors.[8] Studies in animal models of brain injury have shown that galantamine can reduce inflammation after brain injury and offers neuroprotective effects.[9] Thus, galantamine has the potential to reduce SAH-triggered inflammation and improve outcomes. There are several potential mechanisms of action for the efficacy of galantamine to improve outcomes after SAH: 1) Systemic inflammation 2) Central inflammation 3) Improvement in cognition.

1) Vagus nerve, nicotinic α7 receptors and systemic inflammation: The recently described cholinergic anti-inflammatory pathway (CAP) highlights the robust connection between the brain and components of the immune system through the autonomic nervous systems [Figure 1].

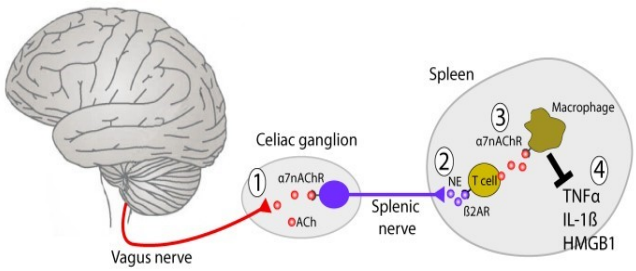


Figure 1. Model for the cholinergic anti-inflammatory pathway. 1) Activation of the efferent component of the vagus nerve releases acetylcholine (ACh) within the celiac ganglion. ACh acts on α7 receptors to enhance splenic nerve activity. 2) Splenic nerve terminals release norepinephrine (NE) within the spleen. NE acts on splenic T lymphocytes causing them to release ACh. 3) ACh activates α7 receptors present on splenic macrophages. 4) Activation of α7 receptors results in decreased pro-inflammatory

Studies have demonstrated that direct stimulation of the vagus nerve (the main efferent arm of the parasympathetic nervous system) decreases serum levels of pro-inflammatory molecules including IL-1ß, TNFα and HMGB-1. Specifically, the α7 receptors present in the celiac ganglion and splenic macrophages are important components of the CAP pathway.[10,11][Figure 1] After

brain injury there is an increase in levels of circulating pro-inflammatory cytokines which correlate with decreases in spleen size suggesting splenic mobilization of splenic macrophages.[12] Dr. Pramod Dash's laboratory has shown that stimulation of the α7 receptors after experimental brain injury decreases both systemic and central inflammation, reduces blood-brain barrier permeability, and offers neuroprotection after traumatic brain injury (Figure 2). Another laboratory has shown similar results in an animal model of SAH.[13]

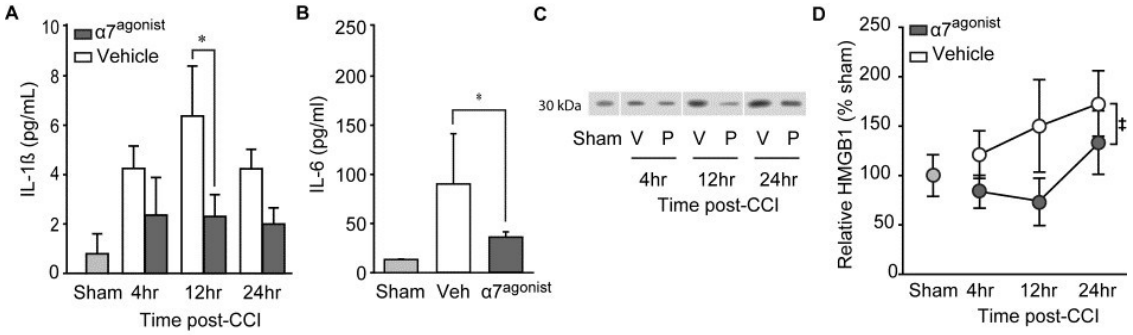


Figure 2. Post-TBI administration of the α7receptor agonist PNU-282987 decreases circulating cytokine, HMGB-1 levels, and reduces the infiltration of inflammatory cells. A) The plasma levels of IL-1ß were found to be significantly decreased at 12 hours post-injury in injured animals treated with the nicotinic Ach receptor α7 agonist PNU-282987 (3 mg/kg i.p.; $\alpha7^{agonist}$) compared to vehicle-treated injured animals (n=5/time point/group). **B)** Compared to the levels detected in vehicle-treated injured animals, rats treated with PNU-282987 had significantly reduced plasma IL-6 levels when assessed 6 hr post-injury (n=3/group). **C)** Representative image of a composite western blot showing HMGB-1 immunoreactivity in the plasma of a sham, injured animals treated with vehicle (V), and injured animals treated with PNU-282987 (P). Images were taken from 3 separate western blots and combined for presentation. Sham samples were run on each membrane and used as a common comparator. **D)** Quantification of the 30kDa HMGB1 band revealed that post-injury administration of PNU-282987 significantly reduced (group main effect by two-way ANOVA) circulating HMGB1 levels (n=5/time point/group).

2) α7 receptors and central inflammation: Unregulated cerebral inflammation is associated with early and delayed brain injury with high levels of inflammatory cytokines in the CSF (including IL-1β, IL-6, IL-8 and TNFα) being associated with poor outcome.[14–19] Several lines of evidence suggest that α7 receptors in the brain modulate cerebral inflammation.[20] For example, in animal models of SAH, increased parasympathetic tone either directly through stimulation of the sphenopalatine ganglion or by activation of perivascular α7 receptor decreases CVSP and improves outcome.[21,22] Furthermore, a decrease in cerebral vasospasm has been associated with decreases in inflammatory markers including IL-6.[23] Ach levels in CSF after SAH are significantly lower in the acute phase of illness, suggesting that reduced Ach receptor activity may play a role in the development of CVSP.[24] Galantamine acts as both an α7 receptor potentiator and an inhibitor of acetylcholinesterase that will increase Ach levels.[25] These two mechanisms of action can work in concert to reduce cerebral inflammation and decrease risk for developing delayed neurologic injury.

3) Nicotinic receptors and cognitive outcome: Cognitive deficit after SAH is a significant cause of morbidity and is associated with an inability to function independently and return to work.[26] As SAH survivors are typically younger than other stroke patients, this places a significant economic burden on family and society. Our preliminary work examining early cognitive deficits have shown that cognitive dysfunction during hospitalization (defined by the modified Montreal Cognitive Assessment Scale (mMoCA)) is associated with worse long term functional outcomes as measured by the modified rankin scale (mRS). Preliminary analysis of 102 SAH patients in our prospective database showed that 1 point decrease on the mMoCA score was associated with a 1.3 times the odds of having a poor outcome (mRS >3) at 6 months when controlled for other clinical variables. Patients with cognitive deficit (defined by a mMoCA score of less than 9) were at a 7.3 times the odds of having a poor clinical outcome at 6 months compared to patients without cognitive deficit.

These findings suggest that early cognitive dysfunction is a target to improve long-term functional outcomes. Galantamine's ability to improve cognitive function in patients with dementia is well established and the mechanism is thought to be from activation of the central nicotinic receptors.[8] The same mechanism may improve early cognitive function after SAH and lead to improvement of functional outcomes.

Galantamine in Neurologic Disease: Galantamine is FDA approved for the treatment of cognitive dysfunction after Alzheimer's disease (AD). It is a potent allosteric potentiating ligand of human nicotinic α7 receptors in the brain. It is also a competitive and reversible cholinesterase inhibitor in all areas of the body. It modulates nicotinic receptors by enhancing Ach activity at the synapse.[25] Multiple randomized, placebo-controlled studies in patients with probable AD have shown that galantamine treatment in range of 16mg to 24mg daily improves cognitive performance.[8,27–29] Galantamine has been shown to be well tolerated and improve cognitive and functional outcomes in patients with vascular dementia and AD with cerebrovascular disease. [30–32] Galantamine is widely used for the treatment of AD and vascular dementia.

In addition to the evidence of safety and efficacy of galantamine for AD, studies have examined the effect of galantamine and similar acetylcholine-esterase inhibitors after acute brain injury. Specifically galantamine was studied in patients with post-stroke aphasia in a pilot randomized clinical trial of 45 patients with chronic post-stroke aphasia. Doses of 8mg twice a day titrated to 16mg twice a day was associated with improvement in aphasia symptoms. [33] Studies have used galantamine in the chronic setting to improved cognitive functioning in traumatic brain injury and ischemic stroke.[34]

Galantamine was studied in patients after traumatic brain injury in the chronic setting to improve cognitive functioning. In an uncontrolled observational study 111 patients were given one of three different types of acetylcholinesterase inhibitors. 30 patients received galantamine. The endpoints examined was a subjective feeling of improvement which was reported by 60% of those receiving galantamine.[34]

A cholinesterase inhibitor, rivastigmine, has been used in the small study in SAH patients. In this study, rivastigmine was used in the chronic stages (at least 9 months after SAH) in patients with persistent cognitive deficits after SAH. They found an improvement in cognitive

function after 12 weeks, but as this was an observational study with no control arm, only speculative conclusions can be made. [35] Our study will be the first to evaluate galantamine (or any other α7 receptor agonist) as a treatment to reduce brain injury in the acute and subacute stages after SAH.

Pharmacokinetics: Previous studies of galantamine have shown bioavailability from oral administration to be 80-100%, with a peak concentration at 1 hour and a half-life of 7 hours. Metabolism is through the liver.[36] The bioavailability of galantamine in SAH patients who are critically ill is not known. However, there is evidence that other medications given orally are absorbed when given in this setting. Nimodipine and pravastatin are both medications which have been given orally after SAH and have demonstrated clinical effects.[37] Although an intravenous formulation might provide easier administration in the acute setting, only an oral formulation is available.

Objectives

Primary Objective:

To investigate the safety and tolerability of galantamine in SAH patients when given 36 hours after admission to hospital and continued for 90 days.

Secondary Objectives:

To develop an evidence-based hypothesis for efficacy of galantamine on the 90 day mRS.

To investigate the effect of galantamine treatment on CSF and serum levels of inflammatory cytokines after SAH.

Study Design

This is a prospective, randomized, placebo-controlled pilot study in adult patients with acute SAH. We will meet all requirements of a clinical trial including protection of human subjects and a three person Data Safety Monitoring Board (DSMB), The study will continue for 3 years or until 60 patients are enrolled. Subjects included will be followed for 90 days.

Primary outcomes:

Primary Tolerability outcome: The ability to stay on study medication

Primary Safety outcome: Death

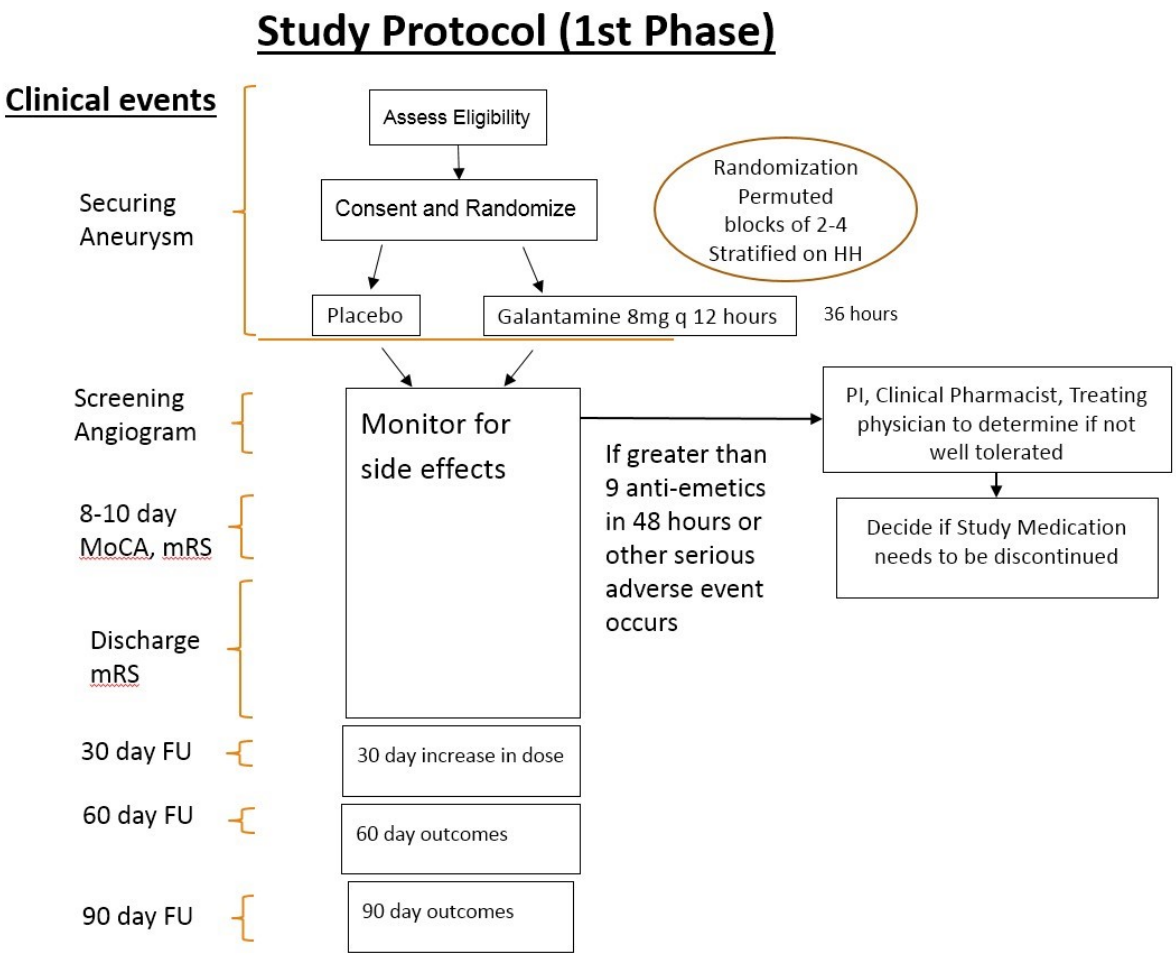
Primary Efficacy outcome: mRS at 90 days used as an ordinal variable

Rate of Improvement in MoCA scores from 1 month to 3 months

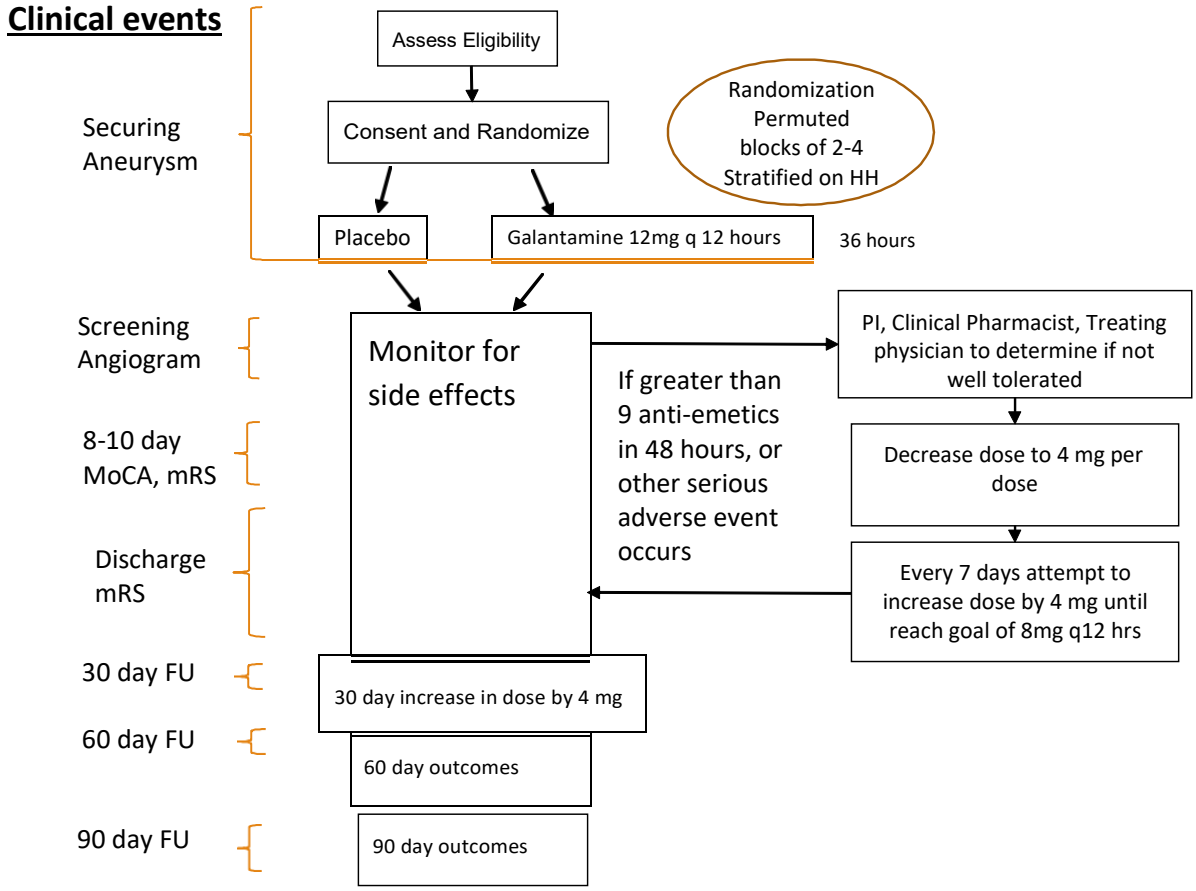
Mechanistic outcome:

Concentrations of inflammatory markers (IL6, IL10, TNFa)

Study Scheme



Study Protocol (2nd Phase)



	Screening/ Randomization (<36 hours)	24-48 hrs	3-5 days	6-8 days	Day 10	Day 30 ± 7 Days	Day 60 ± 7 Days	Day 90 ± 7 Days

Documentation of clinical severity Hunt Hess	**SOC**							
Medical/Treatment History	**SOC**							
NIHSS	**SOC**							
CT head	**SOC**							
CT Angiogram or Conventional Cerebral Angiogram	**SOC**							
Informed Consent	**X**							
T1: Blood Sample/CSF Sample*		**X**						
First dose of medication-continued Q12h	**X**	**X**	**X**	**X**	**X**	**X**		**X**
T2: Blood Sample/CSF Sample*		**X**						
T3: Blood Sample/ CSF Sample*			**X**					
Surveillance Angiography				**SOC**				
MoCA/ mRS					**X**			
T4: Blood Sample/ CSF Sample*				**X**				
Blood Sample/ CSF Sample *§					**X**	**X**	**X**	**X**
Follow Up-Clinic						**X**	**X**	**X**
Urine Pregnancy Test	**SOC**					**X**	**X**	**X**
Outcome Scales (mRS, MoCA, EuroQOL)						**X**	**X**	**X**

SOC = Standard of Care
*** CSF only if available**
§ Day 10 blood sample ≥ 10 days

Study Population

Subjects will be recruited among patients who are admitted to Memorial Hermann Hospital System and the Methodist Hospital System with SAH. The neurosurgical/neurocritical care team and research staff will screen for patients who are eligible. Patients and/or surrogate medical decision makers will be approached by the PI for enrollment into the study.

Inclusion criteria:
1) Spontaneous subarachnoid hemorrhage
2) Presentation to hospital within 72 hours of symptoms
3) Age 18-75
4) Fisher grade 3 hemorrhage (thick subarachnoid clot) on initial CT scan
5) Hunt and Hess grade 1-5 at time of randomization
6) Presence of a cerebral aneurysm on CTA or Angiogram for which clipping or coiling is possible
7) Ability to obtain medication within 36 hours of presentation.

Exclusion criteria:
1) SAH due to causes other than aneurysm rupture (trauma, AVM, mycotic aneurysms, Moyamoya)
2) Pre-existing disability with mRS≥2,
3) Renal disease as defined by creatinine clearance less than 9mL/min
4) History of severe hepatic impairment (Child-Pugh score of 10-15)
5) History of COPD or asthma
6) History of dementia
7) Co-morbid conditions likely to complicate therapy, including clinically significant arrhythmia, AIDS, autoimmune disease, malignancy, and expected mortality within 72 hours
8) Expected mortality within 72 hours as determined by PI, treating neurointensivist and neurosurgeon. (Clinically manifested by no attempt at securing aneurysm)
9) Females who are pregnant.

Study Procedures

This is a pilot study to investigate the safety and tolerability profile of galantamine when given to patients after SAH. Patients admitted to Memorial Hermann Hospital System and the Methodist Hospital System with aneurysmal SAH will be screened. All patients will be cared for in the NSICU as is routine by experienced neurosurgeons and neurointensivists. Those patients or legal surrogates of patients meeting study criteria will be offered consent to participate. All patients enrolled will receive standard of care in adherence to the American Heart Association guidelines for the treatment of SAH patients.[38]

A total of 60 patients will be enrolled. In the first phase subjects will be randomized to galantamine 8mg twice a day or placebo starting within 36 hours and after securing the

aneurysm. After 30 subjects are enrolled, an interim analysis will be performed. Close clinical monitoring will occur to detect potential side effects of the medication. Adverse outcomes will be examined with the Common Terminology Criteria for Adverse Events (CTCAE) Version 4.0. When an adverse event occurs determination of causality will be examined. All serious adverse events meeting Grade 4 criteria will be communicated to the DSMB. All serious adverse events which are unexpected and related will be reported to the DSMB within 7 calendar days of the determination by telephone, email or fax. All other SAEs will be collected and submitted with the report to the DSMB.

Randomization

Eligible patients will be approached as early as possible after presentation to the hospital. Once consented, randomization will be performed by the respective research pharmacy. We plan to randomize using permuted blocks of 2 to 4. The Hunt and Hess Score (HH) is a score based on the neurologic examination the strongest predictor of mortality after SAH. Patients who present with a HH 4 or 5 are at a much higher risk of dying compared to patients who present with a HH1, HH 2 or 3. To account for this we will be stratified based on admission HH (HH2 and 3 versus HH4) to assure that groups are balanced with respect to clinical severity.[39]

Galantamine dose and timing

We will use FDA approved doses of galantamine 8mg every 12 hours in the first phase followed by12 mg every 12 hours in the second phase of our study. Studies in AD patients have shown as low at 16mg daily up to 24mg daily to be associated improved cognitive outcomes. [8] As inflammation after SAH occur early after aneurysmal rupture we are targeting an acute time period of within 36 hours of hospitalization for the beginning of our treatment.[16] Because of the potential increased risk of nausea and vomiting with galantamine, first dose of study medication will be given after securing the aneurysm. All subjects will be treated with placebo or galantamine for 90 days. As the cognitive benefits of galantamine treatment for AD is seen usually about 90 days of treatment we will also use this time frame for treatment after SAH.[8]

In the first phase of the study we will randomize 30 subjects to either galantamine at 8mg every 12 hours or placebo within 36 hours of presentation and after securing the aneurysm. Subjects will be followed for adverse effects while hospitalized which is generally for 14 days. At 30 days and at 60 days subjects will be evaluated for tolerability. An interim analysis will be performed after the first 30 subjects to determine if galantamine at 8mg every 12 hours in the acute setting of SAH is tolerable. Tolerability will be defined by either the subject dropping out of the study because of side effects, the physician discontinuing the medication because of side effects or a difference in serious adverse effects possibly related to the medication.

Once 30 patients are enrolled, if the medication is found to be tolerable (see tolerability section), the second phase of the study we will randomize 30 subjects to either galantamine 12mg every 12 hours or placebo within 36 hours of presentation. Similarly, we will have evaluate tolerability for this dose population.

Potential Adverse Effects

Cardiovascular: Bradycardia and AV block. 2-3% of patients receiving 24 mg daily have been reported to have bradycardia. Our SAH patients are continuously monitored for at least 7 days of hospitalization on continuous telemetry as standard of care.

Gastrointestinal: Increased gastric acid secretion. This may be associated with a higher risk for gastric ulcers. We routinely use histamine blockers or proton pump inhibitors in all our SAH patients to protect against gastric ulcer.

Genitourinary: Urinary retention. This is a frequent problem in the critically ill population. Patients routinely have urinary catheters placed as standard of care to monitor urine output.
Nervous system: Possible seizures. SAH patients are continuously monitored for seizures. In addition all patients are routinely started on antiepileptic medications prophylactically.
Respiratory: Bronchospasm. Respiratory status will be monitored continuously in the Neuroscience intensive care unit (NSICU) for at least the first 7 days and subsequently in the hospital until discharge.
Mortality: In two controlled studies in patients with mild cognitive impairment, the incidence of death in those randomized to receiving galantamine was higher than placebo. The mechanism was unclear and main driver of difference was an unexpectedly low mortality rate in the placebo arm of the studies. [8,40]

Common adverse effects include nausea, vomiting, diarrhea, anorexia, and weight loss. These symptoms are also common after SAH and critical illness. Treatment will be initiated once the aneurysm is secured either through endovascular or surgical clipping of the aneurysm.

This necessitates the need for a blinded study to detect differences between groups.

Monitoring for Adverse Effects

Adverse events will be assessed at all subject visits. Adverse events will be recorded in CRF's with causality assessment.

Clinical follow-up

Demographic information including disease severity on presentation as measured by the HH score, the occurrence of vasospasm on screening angiogram (performed as standard of care) between day 7-8 and the occurrence of DCI will be documented. The MoCA examination will be performed between 8-10 days or within 2 days of discharge. Follow up mRS will be assessed at discharge. At 45 days, subjects will be assessed for tolerability issues

At 90 days, subjects will be assessed with the MoCA examination, EQ5D and mRS. Treatment arm will be unknown to subjects and investigators.

At days 30, 60 and 90, a urine pregnancy test will be administered to women of child bearing potential. Adverse events will be captured at clinic visits (Day 30, Day 60 and Day 90) along with causality assessment.

Outcome measures and statistical analysis

The primary outcome is tolerability (including death) related to galantamine as adjudicated by the DSMB. The primary efficacy outcome will be mRS at 90 days. The modified Rankin focused assessment tool will be used to determine the mRS. If a subject is lost to follow-up we will use the last observation carried forward technique to fill in missing data for the mRS. The MoCA score, and EQ5D at 90 days will be secondary outcomes. The sample size of 60 subjects is the number of patients we believe we can enroll into the study over a 3 year period. We acknowledge the limitations of a small study but plan on using results from this pilot study to launch a larger more definitive study.

Tolerability

Nausea and vomiting are the most likely cause of intolerance to galantamine. However, SAH patients frequently have these symptoms. In fact, the usual clinical presentation for patients with SAH is headache, nausea and vomiting. In a retrospective examination of 141 patients admitted with SAH we found 80% of patients received anti-emetic medications with a median dose requirement of 4 doses (IQR 2-9).

The upper 75% quartile of 9 doses will be used as a screening tool for the detection of possible intolerability of galantamine. If a patient receives more than 9 doses of anti-emetic medications in a 48 hour period we will examine for intolerability. The PI, the treating physician, and the treating clinical pharmacist will determine if dose of medication should be decreased because of persistent nausea and vomiting. If it is decided that the dose should be decreased the dose will be decreased by 4 mg from 8mg to 4 mg or from 12mg to 8 mg. This will occur blinded to treatment allocation. The PI will ask the research pharmacy to decrease the dose and the dose will be decreased whether the treatment allocation is galantamine or placebo.

Once a tolerable dose is found the subject will continue on the dose for a total of 6 doses and an attempt to increase the dose by 4 mg per dose will be made until we reach the target dose of 8 mg twice a day in the first phase or 12mg twice a day in the second phase. If two attempts are made to reach the target dose but tolerability issues persist the subject will remain on the tolerable dose until the end of the 90 day period.

Serum and CSF biomarker analysis

Rationale: Increased inflammation has been associated with worse outcomes after SAH. Our preliminary studies examining subjects from our prospectively collected database show that peripheral inflammation is associated with poor clinical outcome. When examining serum from 87 subjects collected within 48 hours after SAH, we found that inflammatory cytokines IL-6, MCP-1(otherwise known as CCL-2) and TNFα were all significantly elevated in subjects with SAH who progressed to have poor functional outcomes. In multivariable regression analysis IL-6 and MCP-1 were independent predictors of outcome after controlling for known confounders

including age, gender and HH grade. This suggests that IL-6 and MCP-1 may be surrogate markers for poor functional outcomes after SAH. IL-6 is an inflammatory cytokine with broad effects to enhance overall inflammation. It has been shown to be associated with worse clinical outcomes in acute brain injury.18,37 MCP-1 is a chemokine which attracts predominantly monocytes and modulates blood brain barrier permeability.38,39 Elevated levels in CSF have been associated with poor outcome in patients after SAH.38 Studying the effects of galantamine on these cytokines will give an indication whether galantamine has an influence on inflammation after SAH.

In addition to traditional statistical comparisons network analysis techniques can be used to examine the association between multiple variables. The complex nature of cytokine reactions lends itself to this type of analysis. Preliminary studies in our laboratory using an array of 9 cytokines showed differential activation of cytokine networks after SAH between subjects with good and poor outcomes as defined by the mRS at discharge. Specifically we found that patients with poor clinical outcomes had a higher number of significant associations compared to those with good outcomes (18 vs 11) suggesting uncontrolled activation of the inflammatory cascade. Additionally, there was an increase in correlation strengths in all common significant associations in patients with poor outcomes. For instance, in patients with poor discharge outcomes, the IL-6- MCP1β correlation was 78% more compared to patients with good outcomes.

Methods and analysis: Serum and CSF samples will be collected at 8 different predetermined time points. A sample will be collected within 24 hours of admission, (before medication is administered), as a baseline to compare changes in cytokines over time. Samples will be collected between 24-48 hours (after medication is administered), then between 3-5 days, 6-8 days and after 8 days. Levels of inflammatory molecules will be determined by a multiplexed bead array assay platform from BioRad that interrogates the levels of 40 different cytokines and chemokines including IL-6, MCP-1, and TNFα. Samples will be assayed in triplicate and compared to simultaneously prepared standard curves to determine concentrations. Cytokine levels do not typically show a normal distribution, therefore their levels will be compared using the Mann-Whitney U test between subjects who receive galantamine and placebo at each time point. As there can be high variability in cytokine levels, we will use each subject's baseline cytokine levels (before medication) and measure changes in cytokine levels over time. Differences in changes of cytokine at separate time points between the two groups (galantamine and controls) will be compared.

Pairwise Pearson's correlation coefficients will be computed between all pairs of cytokines and a network will be constructed. Networks will be made for subjects who received galantamine and those who received placebo. Networks will be examined for qualitative differences. In addition we will examine for significant differences in the number of associations and differences in strength of associations between those who receive galantamine and controls. A student's t-test and Mann-Whitney U test will be used to test for significant differences in correlation strengths between the networks.

Appropriate measures will be used to control for Type-1 errors common in multiple comparisons. The Benjamani-Hochberg (BH) method with a critical value of 0.1 will be used to adjust p-values to control false discovery rate (FDR). To account for repeated measures over time in cytokine levels per subject mixed effects modeling will be used.41 In addition, we will examine whether there is a dose effect relationship between cytokine levels and galantamine

dose. However, given the small sample size the relationship will be qualitative and not quantitative.

Collection of Specimen

Plasma for biomarker analysis: Blood will be withdrawn from existing lines or by venipuncture and collected into two pink top K_2EDTA 6ml (plasma) Vacutainers per time point (36 ml). The samples will be placed on ice and will be centrifuged at 1,459 X g for 10min at 4°C. The supernatant solution will be removed and centrifuged at 1,459 X g for 10min at 4°C to generate platelet-poor plasma. Plasma will be divided into aliquots and frozen at -80°C until needed. For those subjects providing consent, any residual sample and buffy coat will be frozen at -80°C for future study.

CSF for biomarker analysis: Patients with a ventriculostomy drain will have 1-5 ml of CSF sampled from the receptacle at each of 5 time points. The receptacle will be emptied into the collection bag (the volume will be noted in the nursing notes) to start with an empty receptacle, several drops of CSF will then be allowed to drip into the chamber. The drain will be re-clamped; the chamber port will be cleaned with chlorhexidine as per hospital specifications and the CSF sample drawn from the port with a sterile syringe. CSF will be centrifuged (4°C, 1,459 X g for 10 minutes) and portioned into aliquots and frozen at -80°C; any residual sample will be frozen at -80°C for future study. The CSF pellet should be covered in a 10% DMSO solution (e.g., 100µl CSF plus 11µl DSMO) and frozen at -80°C and transferred to -145°C after 24hrs when possible.

Samples will be labeled with bar-coded subject study identification numbers, sample type and time point. They will be stored indefinitely at the University of Texas Health Science Center at Houston, Neuroscience Research Repository, Hergenroeder Laboratory, The Vivian L. Smith Department of Neurosurgery, 6431 Fannin Street, MSB 7.149, Houston TX 77030

Data and Safety Monitoring

Safety assessments will consist of monitoring and reporting adverse events (AEs), selected adverse events of interest, and serious adverse events (SAEs), both anticipated and unanticipated. An *Adverse Event* is any undesirable and unintended, although not necessarily unexpected, event occurring in human subjects as a result of (a) the interventions and interactions used in the research; or (b) the collection of identifiable private information under the research. *A Serious adverse event* is an adverse event that led to death, or to serious deterioration in the health of the subject, that resulted in death, was life-threatening, required hospitalization (initial or prolonged), resulted in disability or permanent damage, congenital anomaly/birth defect, required intervention to prevent permanent impairment or damage, or other serious events (Important Medical Events). NOTE: Planned hospitalization for a preexisting condition, or a procedure required, without serious deterioration in health, is not considered a serious adverse event. *An unanticipated adverse effect* is defined as any serious adverse effect on health or safety or any life-threatening problem or death caused by, or

associated with, a medication, if that effect, problem, or death was not previously identified in nature, severity, or degree of incidence in the investigational plan or application (including a supplementary plan or application), or any other unanticipated serious problem associated with a medication that relates to the rights, safety, or welfare of subjects.

Once a SAE occurs then an adjudication process will occur to determine if it was possibly related to the study medication. The PI and the treating physician or if the PI is the treating physician, the PI and another blinded board certified neurointensivist will come to a consensus of whether the SAE was possibly and probably related to the study medication.

SAE's that are determined to be unexpected and related will be submitted to the DSMB within 7 calendar days of the determination by telephone, email or fax; written reports will be submitted no later than 15 calendar days of the determination.

Adverse events will be graded according to the USDHHS Common Terminology Criteria for Adverse Events V4.0 (USDHHS, Table 2) and summarized according to body system. Primary focus for analysis will be on tolerability of galantamine and 3-month mortality rate. For the purpose of safety monitoring, adverse events Grade 4 or higher will be classified as important AEs.

Safety/ Stopping Rule

As galantamine is routinely prescribed for patients with dementia, it is likely to be safe in the SAH population as well. In our prospective SAH database, we have a 10% mortality rate in patients. This is a much lower mortality rate compared to other published rates (30-40%) mainly because we will exclude patients who are likely to die imminently.[1] We will use a Bayesian approach of probably of harm to stop the study. Using a previous estimate of death in our patient population of 10% in both arms and a neutral prior with a 95% CI of 0.22-3.0. Using 10,000 simulated trials the probability of stopping incorrectly using the decision rule to stop if there's an 80% probability of increased death in the drug group. The chart below is the stopping rules for the study. Depending on the number of subjects randomized

Num of subjects/arm	Prob of incorrectly stopping	# of excess deaths
6	1%	3
10	3%	3
15	4%	3
20	5%	4
30	8%	4

If stopping rules are met enrollment into the study will be stopped. The DSMB will convene and determine if excess deaths were related to galantamine and will make the determination about continuation of the trial.

A Data and Safety Monitoring Board (DSMB) will be established. The DSMB will include a biostatistician with expertise in research design and an expert in management of brain injured patients. The PI or Co-PI will monitor safety of each enrolled subject. In addition, tolerability will be ongoing with each subject as well as a planned interim analysis. Enrollment will be ongoing during this assessment. Safety for death will occur according to the table provided for the number of excess deaths in the treatment group.

Statistics

Interim analysis:

Interim analysis will be performed after 30 patients are randomized. (15 in each arm). Intolerability will be defined as the need to decrease dose of medication because of side effects, mainly nausea and vomiting. At this time if the galantamine arm has more than 30% intolerability in the first month, defined by dropping out of study because of intolerability, decreasing dose to 4mg every 12 hours because of intolerability or difference in SAEs, compared to the placebo arm, in the second phase of the study we will decrease the starting dose to 4 mg every 12 hours and attempt to increase after 7 days to 8 mg every 12 hours.

	Placebo (N=15)	Active (N=15)	Action
% of subjects requiring dose decreases	X	Less than X+30	Continue to a goal of 12mg q12
		Greater than X+30	Decrease starting dose to 4mg q12

The enrollment will be held only for assessment of intolerability from the first month of galantamine as side effects and intolerability is likely to occur during initiation of therapy.

The primary efficacy endpoint will be the mRS used as an ordinal variable (5 and 6 will be grouped). Analysis will be stratified based on HH grade. All results will be reported as Relative Risks with 95 CI. The estimates of relative risk found in this study will be used for future meta-analysis or planning for the next phase clinical trial. In the larger study, utilizing a Bayesian approach, the posterior estimate and 95% CrI could be utilized as prior estimates for the larger confirmatory trial. Secondary outcomes including the mMoCA performed between 14-21 days or within 2 days of discharge, MoCA scores at 30, 60 and 90 days, and the EQ5D at 30, 60 and 90 days will be also be reported similarly.

Ethics

This protocol and the informed consent document and any subsequent modifications will be reviewed and approved by the University of Texas Health Science Center at Houston IRB. A signed consent form will be obtained for every subject either by the subject or a LAR if the patient is not able to consent. The consent form will describe the purpose of the study, the procedures to be followed, and the risks and benefits of participation. A copy of the consent form will be given to the patient or LAR.

Data handling and record keeping

Study personnel will collect information on subject characteristics such as demographics, medical history, and other clinical information. The initial assessment of the subject will be recorded including physical and neurological examination, vital signs, laboratory tests, and radiographic imaging. In addition, as part of the baseline data collection, family members will be asked to complete a contact information form with the names, addresses and phone numbers of other people who could assist in locating him/her if the study coordination cannot locate him/her for the scheduled phone interviews. The study will consider the use of a locator service for patients potentially lost to follow-up.

Additional CRFs will be generated to record disease and injury-related events, treatments and interventions during the initial hospitalization, and safety data for adverse event reporting.

Federal law now holds the statute of limitations at six years to bring forward an allegation of research misconduct. In response to this extension, research records must be retained for a sufficient period to investigate an allegation of research misconduct - - a minimum period of six years. Additionally, existing Federal regulations [56 CFR 56.115(b)] require that IRB records be retained for at least 3 years after completion of the research. All records must be accessible for inspection and copying by authorized representatives of HHS and Food and Drug Administration at reasonable times and in a reasonable manner. At the end of the three year period, the IRB records may be boxed, labeled and sent to central storage for an additional 3-10 years. A log of stored records is maintained in the IRB office for retrieval if files are needed for audit or other purposes.

Records will be maintained in a de-identified manner in a secure, locked location to ensure confidentiality. Subjects will not be identified in any manner in any publications resulting from this project.

De-identified data will be collected from the pre-hospital period through 6 months post injury. A linking list will be maintained by the investigative team at each site to allow for patient follow-up. Protected health information (PHI) is confidential and not shared outside the study team. Written data will be stored in locked areas; electronic data will be password protected in secure zones. Patient name and contact information will be separated from subject data. The PI will monitor protocol compliance and data integrity. Routine data audits will be conducted internally to assure consistency and integrity of the data. Additionally, an independent data auditor will verify data quality.

Quality control and assurance

De-identified data will be collected from the pre-hospital period through 3 months post SAH. A linking list will be maintained by the investigative team at each site to allow for patient follow-up. Protected health information (PHI) is confidential and not shared outside the study team. Written data will be stored in locked areas; electronic data will be password protected in secure zones. Patient name and contact information will be separated from subject data. The PI will monitor protocol compliance and data integrity. Routine data audits will be conducted internally to assure consistency and integrity of the data.

Data will be linked by unique ID. The study statistician will independently review the forms and the entered data. The database will incorporate quality control checks at data entry, and quality assurance programs will be written for acquisition, management, tracking and retrieval and will be run regularly. The database will be backed up onto separate media. The database is maintained for statistical analysis purpose only; thus, all electronic files will be de-identified. The database will be password protected and maintained in secure research offices in a secure research building. Data validation checks identifying outliers will generate queries which will be evaluated and if necessary returned to the clinical study site for data validation. An audit trail will be maintained on changes to the database.

Database queries will be designed and implemented to oversee compliance with all study documentation and timely completion of CRFs. Protocol deviations, record completion, and regulatory compliance will be reviewed the PI. All study documents and pertinent records will be available for inspection by the DSMB or other designated monitoring authority.

Publication Plan

The study will be reported on clinicaltrials.gov and other scientific medical journals. The results will not be returned to specific research subjects.

ATTACHMENTS

1. Schematic of Study Design
2. Study Schedule
3. Consent Document
4. Case Report Form

Bibliography

1. Lovelock CE, Rinkel GJ, Rothwell PM. Time trends in outcome of subarachnoid hemorrhage: Population-based study and systematic review. Neurology 2010;74(19):1494–501.

2. Taylor TN, Davis PH, Torner JC, Holmes J, Meyer JW, Jacobson MF. Lifetime cost of stroke in the United States. Stroke [Internet] 1996 [cited 2015 Nov 21];27(9):1459–66. Available from: http://www.ncbi.nlm.nih.gov/pubmed/8784113

3. Sehba F a, Pluta RM, Zhang JH. Metamorphosis of subarachnoid hemorrhage research: from delayed vasospasm to early brain injury. Mol Neurobiol [Internet] 2011 [cited 2013 Oct 25];43(1):27–40. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3023855&tool=pmcentrez&rendertype=abstract

4. Macdonald RL. Delayed neurological deterioration after subarachnoid haemorrhage. Nat Rev Neurol [Internet] 2013;10(1):44–58. Available from: http://www.nature.com/doifinder/10.1038/nrneurol.2013.246

5. Sehba F a, Bederson JB. Mechanisms of acute brain injury after subarachnoid hemorrhage. Neurol Res [Internet] 2006;28(4):381–98. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16759442

6. Friedrich V, Flores R, Muller A, Bi W, Peerschke EI, Sehba F a. Reduction of neutrophil activity decreases early microvascular injury after subarachnoid haemorrhage. J Neuroinflammation [Internet] 2011 [cited 2013 Oct 25];8(1):103. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3170601&tool=pmcentrez&rendertype=abstract

7. Sussman ES, Kellner CP, McDowell MM, et al. Alpha-7 nicotinic acetylcholine receptor agonists in intracerebral hemorrhage: an evaluation of the current evidence for a novel therapeutic agent. Neurosurg Focus [Internet] 2013;34(5):E10. Available from: http://eutils.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?dbfrom=pubmed&id=23634914&retmode=ref&cmd=prlinks\npapers2://publication/doi/10.3171/2013.2.FOCUS1315

8. Loy C. SL. Galantamine for Alzheimer ' s disease and mild cognitive impairment. Cochrane Libr 2009;(1):1–3.

9. Pavlov V a., Parrish WR, Rosas-Ballina M, et al. Brain acetylcholinesterase activity controls systemic cytokine levels through the cholinergic anti-inflammatory pathway. Brain Behav Immun [Internet] 2009;23(1):41–5. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0889159108003000

10. Rosas-Ballina M, Tracey KJ. The Neurology of the Immune System: Neural Reflexes Regulate Immunity. Neuron [Internet] 2009;64(1):28–32. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0896627309007508

11. Wang H, Yu M, Ochani M, et al. Nicotinic acetylcholine receptor alpha7 subunit is an essential regulator of inflammation. Nature 2003;421(6921):384–8.

12. Walker P a, Shah SK, Jimenez F, et al. Intravenous multipotent adult progenitor cell therapy for traumatic brain injury: preserving the blood brain barrier via an interaction with splenocytes. Exp Neurol [Internet] 2010 [cited 2014 Jun 10];225(2):341–52. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3774549&tool=pmcentrez&rendertype=abstract

13. Duris K, Manaenko A, Suzuki H, Rolland WB, Krafft PR, Zhang JH. ??7 nicotinic acetylcholine receptor agonist PNU-282987 attenuates early brain injury in a perforation model of subarachnoid hemorrhage in rats. Stroke 2011;42(12):3530–6.

14. Dumont AS, Dumont RJ, Chow MM, et al. Cerebral Vasospasm after Subarachnoid Hemorrhage: Putative Role of Inflammation. Neurosurgery [Internet] 2003 [cited 2013 Oct 25];53(1):123–35. Available from: http://content.wkhealth.com/linkback/openurl?sid=WKPTLP:landingpage&an=00006123-200307000-00016

15. Hanafy K a, Grobelny B, Fernandez L, et al. Brain interstitial fluid TNF-alpha after subarachnoid hemorrhage. J Neurol Sci [Internet] 2010 [cited 2013 Oct 24];291(1-2):69–73. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2834870&tool=pmcentrez&rendertype=abstract

16. Chou SH-Y, Feske SK, Atherton J, et al. Early elevation of serum tumor necrosis factor-α is associated with poor outcome in subarachnoid hemorrhage. J Investig Med [Internet] 2012 [cited 2013 Oct 25];60(7):1054–8. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3740211&tool=pmcentrez&rendertype=abstract

17. Singh N, Hopkins SJ, Hulme S, et al. The effect of intravenous interleukin-1 receptor antagonist on inflammatory mediators in cerebrospinal fluid after subarachnoid haemorrhage: a phase II randomised controlled trial. J Neuroinflammation [Internet] 2014;11(1):1–8. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3892121&tool=pmcentrez&rendertype=abstract

18. Hopkins SJ, McMahon CJ, Singh N, et al. Cerebrospinal fluid and plasma cytokines after subarachnoid haemorrhage: CSF interleukin-6 may be an early marker of infection. J Neuroinflammation [Internet] 2012;9(1):255. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3526412&tool=pmcentrez&rendertype=abstract

19. Provencio JJ, Fu X, Siu a, Rasmussen P a, Hazen SL, Ransohoff RM. CSF neutrophils are implicated in the development of vasospasm in subarachnoid hemorrhage. Neurocrit Care [Internet] 2010 [cited 2013 Oct 25];12(2):244–51. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2844469&tool=pmcentrez&rendertype=abstract

20. Shytle RD, Mori T, Townsend K, et al. Cholinergic modulation of microglial activation by α7 nicotinic receptors. J Neurochem 2004;89:337–43.

21. Si M-L. alpha7-Nicotinic Acetylcholine Receptors on Cerebral Perivascular Sympathetic Nerves Mediate Choline-Induced Nitrergic Neurogenic Vasodilation. Circ Res [Internet] 2002;91(1):62–9. Available from: http://circres.ahajournals.org/cgi/doi/10.1161/01.RES.0000024417.79275.23

22. Yarnitsky D, Lorian A, Shalev A, et al. Reversal of cerebral vasospasm by sphenopalatine ganglion stimulation in a dog model of subarachnoid hemorrhage. Surg Neurol [Internet] 2005;64(1):5–11; discussion 11. Available from: http://www.ncbi.nlm.nih.gov/pubmed/15993169

23. Song J-N, Zhang M, Li D-D, et al. Dynamic expression of the suppressor of cytokine signaling-3 and cytokines in the cerebral basilar artery of rats with subarachnoid hemorrhage, and the effect of acetylcholine. Acta Neurochir (Wien) [Internet] 2014 [cited 2015 Nov 5];156(5):941–9; discussion 949. Available from: http://www.ncbi.nlm.nih.gov/pubmed/24463741

24. Kawamata T, Takeshita M, Ujiie H, et al. Acetylcholine determination of cerebrospinal fluid in aneurysmal subarachnoid hemorrhage. Surg Neurol [Internet] 1994 [cited 2015 Nov 5];41(5):399–404. Available from: http://www.ncbi.nlm.nih.gov/pubmed/8009415

25. Samochocki M, Höffle A, Fehrenbacher A, et al. Galantamine is an allosterically potentiating ligand of neuronal nicotinic but not of muscarinic acetylcholine receptors. J Pharmacol Exp Ther 2003;305(3):1024–36.

26. Wong GKC, Lam SW, Ngai K, et al. Cognitive domain deficits in patients with aneurysmal subarachnoid haemorrhage at 1 year. J Neurol Neurosurg Psychiatry [Internet] 2013 [cited 2015 Nov 5];84(9):1054–8. Available from: http://jnnp.bmj.com.ezproxyhost.library.tmc.edu/content/84/9/1054.long

27. Raskind MA, Peskind ER, Wessel T, Yuan W. Galantamine in AD: A 6-month randomized, placebo-controlled trial with a 6-month extension. Neurology [Internet] 2000;54(12):2261–8. Available from: Available from Neurology in http://link.worldcat.org/?jHome=http://www.nelft.nhs.uk/services/library&linktype=best

28. Tariot PN, Solomon PR, Morris JC, Kershaw P, Lilienfeld S, Ding C. A 5-month, randomized, placebo-controlled trial of galantamine in AD. Neurology 2000;54(12):2269–76.

29. Rockwood K, Mintzer J, Truyen L, Wessel T, Wilkinson D. E V ects of a flexible galantamine dose in Alzheimer ' s disease : a randomised , controlled trial. 2001;589–95.

30. Erkinjuntti T, Kurz A, Gauthier S, Bullock R, Lilienfeld S, Damaraju C V. Efficacy of galantamine in probable vascular dementia and Alzheimer's disease combined with cerebrovascular disease: a randomised trial.[comment]. Lancet 2002;359(9314):1283–90.

31. Kurz AF, Erkinjuntti T, Small GW, Lilienfeld S, Damaraju CRV. Long-term safety and cognitive effects of galantamine in the treatment of probable vascular dementia or Alzheimer's disease with cerebrovascular disease. Eur J Neurol 2003;10(6):633–40.

32. Erkinjuntti T, Gauthier S, Bullock R, et al. Galantamine treatment in Alzheimer's disease with cerebrovascular disease: responder analyses from a randomized, controlled trial

(GAL-INT-6). J Psychopharmacol 2008;22(7):761–8.

33. Hong JM, Shin DH, Lim TS, Lee JS, Huh K. Galantamine administration in chronic post-stroke aphasia. J Neurol Neurosurg Psychiatry [Internet] 2012;83(7):675–80. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22595363

34. Tenovuo O. Central acetylcholinesterase inhibitors in the treatment of chronic traumatic brain injury-clinical experience in 111 patients. Prog Neuropsychopharmacol Biol Psychiatry 2005;29(1):61–7.

35. Wong GK-C, Wong R, Mok V, et al. Rivastigmine for cognitive impairment after spontaneous subarachnoid haemorrhage: a pilot study. J Clin Pharm Ther [Internet] 2009;34(6):657–63. Available from: http://www.ncbi.nlm.nih.gov/pubmed/20175798

36. Colović MB, Krstić DZ, Lazarević-Pašti TD, Bondžić AM, Vasić VM. Acetylcholinesterase inhibitors: pharmacology and toxicology. Curr Neuropharmacol [Internet] 2013;11(3):315–35. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3648782&tool=pmcentrez&rendertype=abstract

37. Tseng M-Y, Hutchinson PJ, Turner CL, et al. Biological effects of acute pravastatin treatment in patients after aneurysmal subarachnoid hemorrhage: a double-blind, placebo-controlled trial. J Neurosurg [Internet] 2007;107(6):1092–100. Available from: http://eutils.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?dbfrom=pubmed&id=18077945&retmode=ref&cmd=prlinks\npapers2://publication/doi/10.3171/JNS-07/12/1092

38. Connolly ES, Rabinstein AA, Carhuapoma JR, et al. Guidelines for the management of aneurysmal subarachnoid hemorrhage: a guideline for healthcare professionals from the American Heart Association/american Stroke Association. Stroke [Internet] 2012 [cited 2015 Sep 12];43(6):1711–37. Available from: http://www.ncbi.nlm.nih.gov/pubmed/22556195

39. Doig GS, Simpson F. Randomization and allocation concealment: a practical guide for researchers. J Crit Care [Internet] 2005;20(2):187–91. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0883944105000420

40. B. W, S. G, L. S, et al. Safety and efficacy of galantamine in subjects with mild cognitive impairment. Neurology [Internet] 2008;70(22):2024–35. Available from: http://ovidsp.ovid.com/ovidweb.cgi?T=JS&PAGE=reference&D=emed8&NEWS=N&AN=2009284614