Augmenting Cerebral Blood Flow to Treat Established Multiple Sclerosis

NCT02466074

Version Date: 06/27/2019

STUDY PROTOCOL

PROTOCOL NUMBER:
HSC-MS-14-0450

PROTOCOL TITLE:
Augmenting Cerebral Blood Flow to Treat Established Multiple Sclerosis

VERSION: 6.0
DATE: April 1, 2019

CORRESPONDING PRINCIPAL INVESTIGATOR:
John A. Lincoln, MD, PhD, Associate Professor, UTHealth

SITE PRINCIPAL INVESTIGATOR:
John A. Lincoln, MD, PhD, Associate Professor, UTHealth

SITE:
McGovern Medical School, UTHealth

COLLABORATORS:
Léorah Freeman, MD, Assistant Professor, UTHealth
Khader Hasan, PhD, Professor, UTHealth
Refaat Gabr, PhD, Assistant Professor, UTHealth
Jerry Wolinsky, MD, Emeritus Professor, UTHealth
Ponnada Narayana, PhD, Professor, UTHealth

GENERAL INFORMATION

Name and address of the person authorized to sign the protocol and amendments:
John A. Lincoln, MD, PhD, [redacted]

Name and address of study monitor:
James R. Jemelka, [redacted]

Name, title, address and telephone number(s) of the medical expert for the trial
John A. Lincoln, MD, PhD, [redacted]
, 713-500-7016

Name and title of the investigator(s) and sub-investigators responsible for the trial with address and phone number(s)
John A. Lincoln, MD, PhD, [redacted]
, 713-500-7016

Name and addresses of the clinical laboratories and/or other institutions involved in the trial
N/a

TABLE OF CONTENTS

STUDY PROTOCOL

BACKGROUND AND RATIONALE

General Introduction

Multiple sclerosis (MS) is a chronic central nervous system disease affecting over 400,000 persons in the United States and over 2.5 million persons worldwide (Reingold, 2002). Clinical symptoms typically occur as a result of focal inflammation associated with demyelination and axonal damage. Symptoms can be transient for many patients during the initial stage of disease with some degree of improvement as inflammation subsides and tissue undergoes repair (Lassmann, 2011; Leary, 2005). Additionally, there is mounting evidence that inflammation might not be a prerequisite for tissue injury (Barnett et al., 2006; Barnett and Prineas, 2004; Narayana et al., 1998). Regardless of how tissue is initially injured, the degree of repair and corresponding resolution of clinical symptoms is often variable and not entirely understood.

Magnetic resonance imaging (MRI) is commonly utilized to monitor lesion formation and evolution (Cotton et al., 2003; Meier and Guttmann, 2006; Narayana et al., 1998). The majority of new MS plaques initially enhance after administration of intravenous (IV) contrast, a hallmark of acute perivascular inflammation. These new plaques appear bright (hyperintense) on T2-weighted and frequently have a corresponding dark component (hypointense) on T1-weighted sequences (Cotton et al., 2003; Filippi, 2000; Meier and Guttmann, 2006) [see figure 1]. Within months from their initial appearance as newly formed lesions, as much as 80% of the initial T2 lesion volume resolves largely related to resolution of edema and partial tissue repair (Cotton et al., 2003). By contrast, up to 40% of lesions with initial T1 hypointense components will evolve into persistent black holes (chronic area with low signal intensity on T1-weighted images) indicative of permanent tissue damage (Sahraian et al., 2010). Many studies have demonstrated a robust correlation between persistent black holes and clinical disability (Parry et al., 2002; Thaler et al., 2017).

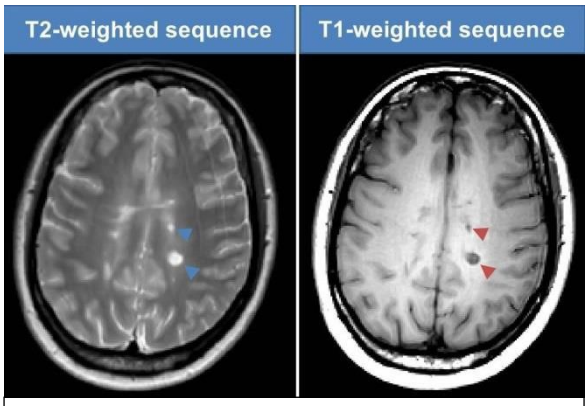


Figure 1: MS lesion appears as hyperintense (blue arrows) on T2-weighted and hypointense (red arrows) on T1-weighted sequences respectively.

There are regional differences in cerebral perfusion within the normal human brain. Cerebral perfusion is altered in many disease states, including MS. Altered perfusion has been seen in patients with all MS phenotypes and early in relapsing-remitting disease (Helenius et al., 2003; Papadaki et al., 2013, 2012; Wuerfel et al., 2004). Changes in cerebral perfusion both in a given region and lesion area have been previously described in multiple sclerosis (Juurlink, 2013; Wuerfel et al., 2004). Our own work is consistent with others showing that areas within the brain that are most prone to hypoperfusion appear to be those with minimal collateral flow (Adhya et al., 2006; Helenius et al., 2003; Holland et al., 2012; Juurlink, 2013; Narayana et al., 2014; Wuerfel et al., 2004). It is noteworthy that studies, including our own, have shown increased probability of lesion formation within areas corresponding to expected hypoperfused regions (Debernard et al., 2013; Holland et al., 2012; Narayana et al., 2014). Diminished regional perfusion and increased energy demand has also been suggested to potentiate axonal loss within lesions and contributes to the evolution of potentially irreversible and progressive tissue injury, visualized on serial MRI as persistent black holes (Trapp and Stys, 2009). Indeed, these changes appear to be dynamic and

associated with lesion evolution (Wuerfel et al., 2004).

There are currently several potent anti-inflammatory medications available to treat patients with MS. All of these disease modifying therapies (DMTs) decrease the potential for new injury to varying degrees. Despite their anti-inflammatory properties, DMTs are not known to enhance cerebral perfusion. There are several medications currently available that are known to transiently enhance cerebral perfusion. Of these, acetazolamide (ACZ) is well tolerated and has a well-established safety profile ("Acetazolamide Tablets | Drug Summary | PDR.net," n.d.; Lim et al., 2001; Stewart et al., 2002). Computerized tomography (CT) or MRI perfusion imaging with IV ACZ challenge is a commonly utilized clinical tool in evaluating patients with chronic intracerebral occlusive disease and is a reliable predictor of critically reduced cerebral perfusion (Vagal et al., 2009). Acetazolamide is also currently utilized as an IV drug in the treatment of acute, closed-angle glaucoma, drug-induced edema or congestive heart failure and as an oral drug taken chronically in divided daily doses as adjunct treatment of chronic open-angle glaucoma, epilepsy, pseudotumor cerebri, chronic congestive heart failure, acute motion sickness, uric acid renal calculi and familial periodic paralyses.

If cerebral hypoperfusion is important to the evolution of lesions then encouraging better tissue perfusion might reduce the evolution and persistence of black holes.

Rationale and justification for the Study

Rationale for the Study Purpose

Focal regions of demyelination, variable axonal loss and gliosis characterize the pathologic damage that is the hallmark of MS. These lesions appear as T2 hyperintense when imaged by MRI. Diminished regional perfusion and increased energy demand within the MS lesion have been suggested to potentiate axonal loss. Axonal damage is often seen on MRI as an area of T1 hypointensity ("black hole") within the T2 hyperintense lesion (van Walderveen et al., 1998, 1999a). Indeed up to 90% of axons might be damaged within the area imaged as black hole (van Walderveen et al., 1999a). Several reports have shown stronger correlations in MS patients between both general and system-specific clinical disability and black hole volume as compared to total T2 hyperintense lesion volume (Caramanos et al., 2012; Giorgio et al., 2013; van Walderveen et al., 1999a).

There are twelve DMTs currently approved by the Food and Drug Administration (FDA) to treat patients with MS. All of these therapies impact the immune system and, in the aggregate, have been shown to decrease new inflammatory lesions (T2 hyperintense lesions) [see review by Tullman; (Tullman, 2013)]. Though we are able to decrease the potential for new injury, there are no medications that are shown to decrease the likelihood of black hole formation (lesion evolution) once the T2 lesion forms. If cerebral perfusion is an important contributor to tissue repair then MS lesions within hypoperfused areas might be more likely to develop permanent tissue loss. In this scenario, medications that encourage cerebral perfusion might alter the evolution of new MS plaques and diminish clinical disability progression.

To test this hypothesis, we propose to utilize ACZ, an existing medication with a well-established safety and tolerability profile that has been shown to enhance cerebral perfusion ("Acetazolamide Tablets | Drug Summary | PDR.net," n.d.; Lim et al., 2001; Stewart et al., 2002). To the best of our knowledge, ACZ has not been previously evaluated as a potential therapy in patients with MS. This study will determine if the novel application of long-term ACZ therapy in subjects with MS improves cerebral perfusion resulting in a smaller proportion of MS lesions that evolve to form the persistent black holes that reflect permanent tissue damage. Stage 1a of the study will define the magnitude of change in regional cerebral perfusion following acute IV ACZ therapy. Stage 1b will determine the degree to which short-term use of divided daily oral doses of ACZ improves global and regional

cerebral perfusion in subjects with MS. Finally, Stage 2 of the study will determine the effect of long-term oral ACZ on lesion evolution.

Rationale for Doses Selected

Studies have shown that ACZ administered as a single 1000 mg IV bolus transiently enhances cerebral perfusion in healthy subjects (Sullivan et al., 1987; Vagal et al., 2009). Subjects challenged with IV ACZ had between a 30% to 60% increase in cerebral blood flow, as measured by Xenon-enhanced computed tomography scan, within 15 minutes of IV bolus (Sullivan et al., 1987). Another study using ultrasonography demonstrated that enhanced perfusion was sustained for at least 30 minutes with return to baseline measures within one hour (Hartkamp et al., 2012). This same dose and route of administration will be used in subjects with MS to determine the transient change to cerebral perfusion as quantified using MRI metrics as described in section 2.2.

Though ACZ IV transiently enhances cerebral perfusion, longer-term changes would be required to potentially impact lesion evolution. Daily or divided-daily infusions necessitate chronic IV access, pose increased risk to the patient and are impractical. Oral ACZ has been shown to also increase cerebral perfusion in patients with other neurologic disorders ("Acetazolamide Tablets | Drug Summary | PDR.net," n.d.) and might prove a practical and effective alternate chronic therapy for patients with MS.

ACZ has been used in daily divided doses up to a maximum of 4000 mg per day to treat pseudotumor cerebri (NORDIC Idiopathic Intracranial Hypertension Study Group Writing Committee et al., 2014). Participants in Stage 1b of this study will be given escalating divided doses of oral ACZ up to 1500 mg per day to determine both the magnitude of change to cerebral perfusion with each dose and define the optimum dose(s) for additional studies. In the final stages of this study (stage 2a and 2b), subjects will be treated with ACZ, as determined in stage 1b, for six consecutive months to determine if long-term change to cerebral perfusion alters the evolution of MS plaques into persistent black holes.

Rationale for Study Population

Multiple sclerosis affects predominantly persons between 15 and 55 years of age with females being nearly four-times as likely to be diagnosed with disease as males (Koch-Henriksen and Sørensen, 2010; Orton et al., 2006; Reingold, 2002). MS in the paediatric population is now better recognized and has been shown to differ from that in the adults in both clinical and radiologic presentation and evolution (Ghassemi et al., 2014; Renoux et al., 2007).

This study will utilize female and male MS subjects between 18 and 55.

Rationale for Study Design

This study is designed as a prospective trial using a delayed-start design which is anticipated to lead to a larger randomized phase 3 clinical trial. Subjects will be evaluated prior to and after initiation of therapy to quantify the effect of therapy on lesion evolution. These initial pilot studies will determine the magnitude to which ACZ improves MRI measures of cerebral perfusion and alters the evolution of MS plaques. The studies outlined in this proposal will be useful not only in power calculation for the anticipated larger randomized clinical trial but also determine imaging characteristics of responders and non-responders, as it relates to treatment effect and identify the optimum outcome measures, including frequency at which measurements should be obtained.

Many factors contribute to the formation and evolution of lesions in MS (Cotton et al., 2003; Meier and Guttmann, 2006; Narayana et al., 1998). As much as 60% of MS lesions resolve over six months while the remainder show evidence of persistent damage (Sahraian et al., 2010). In stage 2, MS subjects will be treated with oral ACZ for 24 and 48 consecutive weeks to determine if improved cerebral perfusion will result in improved tissue integrity within lesions.

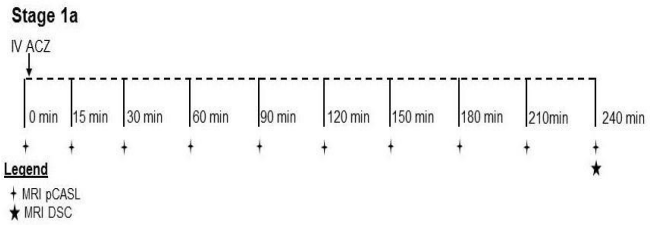
The formation of new MS lesion is best visualized on contrast-enhanced T1-weighted sequences and usually corresponds to new hyperintense area on T2-weighted MRI. To evaluate both existing and newly formed MS lesion, subjects will obtain both contrast-enhanced and non-contrast MRI.

Stage 1a - Increases in cerebral perfusion after IV ACZ has been seen in healthy persons but not in subjects with MS. In addition to physiologic differences in regional cerebral perfusion, subjects with MS might have exaggerated differences secondary to disease-associated damage. This stage will determine the degree of change, temporal kinetics and difference between various regions in cerebral perfusion after IV ACZ in subjects with MS.

Arterial spin labeling (ASL) is a non-invasive MRI technique where spins are tagged using a radio-frequency pulse to determine cerebral blood flow (CBF). Both continuous (CASL) and pulsed arterial spin (PASL) techniques have been utilized to measure CBF. CASL has the advantage of improved signal-to-noise and provides a clearer estimate of CBF though the technique is longer with greater demands on imaging hardware. PASL has the advantage of higher tagging efficiency and is a shorter technique though with greater associated noise. Pseudo-continuous ASL (pCASL) is a more recent technique that combines the merits of both CASL and PASL and largely overcomes previous disadvantages (Dai et al., 2008; Wong, 2007; Wu et al., 2007). pCASL technique has recently been further optimized to accurately and reproducibly quantify CBF (Aslan et al., 2010). Our lab has previously published on the use of pCASL to determine CBF in MS subjects (Narayana et al., 2014).

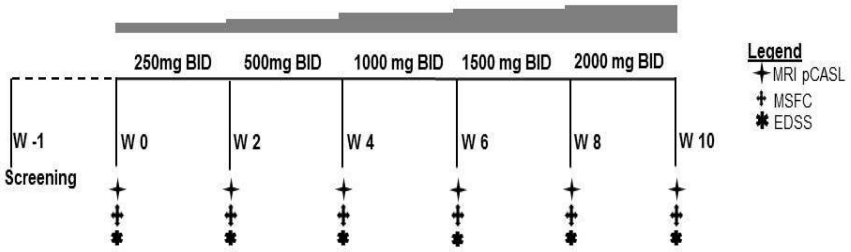
Dynamic susceptibility contrast (DSC) MRI is a gadolinium contrast-based technique that can accurately and reproducibly quantify cerebral blood flow (CBF), cerebral blood volume (CBV) and mean transit time (MTT) of blood. There is a very small risk of an anaphylactic reaction to the injection of all commercially available paramagnetic contrast agents for the use in diagnostic and research imaging. There is a risk of the development of nephrogenic systemic fibrosis (NSF) following the administration of some gadolinium containing contrast agents to patients with moderate to severe renal disease and those on dialysis. Following recent recommendations of the American College of Radiology (see Kanal et al. AJR 188:1447, 2007), patients with renal disease are excluded from these studies. Standard precautions available for the management of such reaction are available within the UT Imaging Center where these studies will be performed. DSC techniques will provide information that cannot be obtained by using pCASL alone that is important to determine how blood flow is altered with ACZ.

The degree of transient change to CBF, measured using pCASL, will be determined in MS subjects prior to and after administration of a 1000 mg IV bolus of ACZ. Time to peak effect and duration of action (not previously defined in MS subjects) will be monitored using pCASL at 15, 30, 120 and 240 minutes from time of infusion. At the end of the kinetic study gadolinium will be injected and DSC measures of blood flow obtained.



Stage 1b - Changes in cerebral perfusion will likely need to be sustained for prolonged periods to impact lesion evolution. Participants in this stage of the study will be given escalating divided doses of oral ACZ up to 4000 mg per day to determine both the magnitude of change to cerebral perfusion with maintenance daily oral ACZ therapy and to define the optimum dose(s) for additional studies.

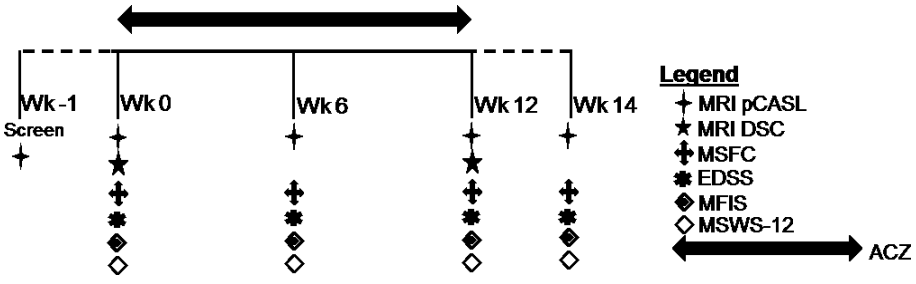
Subjects will undergo MRI using pCASL prior the start of each bi-monthly regimen of ACZ as well as on the day of drug discontinuation. The magnitude of change in CBF will be determined for various oral ACZ doses as well as for various cerebral regions



Stage 2a –Safety/Tolerability & Kinetics of Treatment Effect

This stage will continue to gather information about whether daily oral ACZ is tolerated and the kinetics of the treatment effect. Patients continue on the same dose for 12 consecutive weeks and undergo repeat imaging at predetermined intervals. Sustained treatment over a short duration will allow us to rapidly determine whether sustained treatment with ACZ can alter cerebral blood flow and will provide a go/no-go for future studies using this drug/paradigm. In this stage, all subjects will receive baseline pCASL and DSC MRI, will start oral ACZ continued for 12 consecutive weeks and undergo repeat imaging at predetermined intervals. MRI scans and clinical evaluations will be obtained every 6 weeks with contrast-based scans repeated at 12 weeks. As previously stated, DSC provides measurements of not only CBF but also blood volume (CBV) and transit time of blood (MTT). It is possible that in addition to changes in blood flow, the amount or rate of flow is altered with ACZ and may be an important mechanism to tissue repair. DSC techniques will provide information that cannot be obtained by using pCASL alone that might be important to determine how blood flow is altered with ACZ.

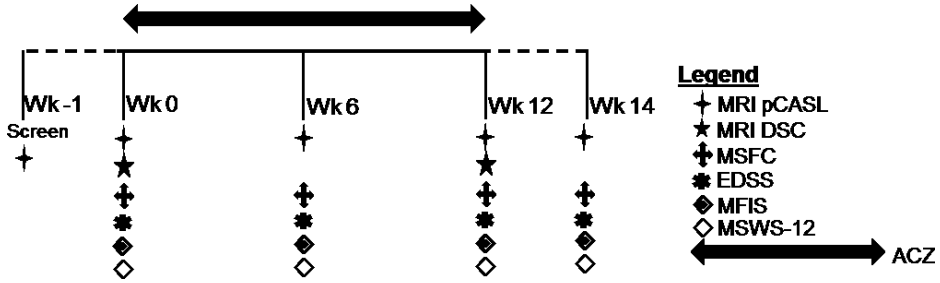
In addition, we will use advanced MRI techniques to also quantify tissue oxygen extraction before and after ACZ therapy as well as determine the metabolic rate of oxygen consumption in various tissues.



Stage 2a –Safety/Tolerability & Kinetics of Treatment Effect

This stage will continue to gather information about whether daily oral ACZ is tolerated and the kinetics of the treatment effect. Patients continue on the same dose for 12 consecutive weeks and undergo repeat imaging at predetermined intervals. Sustained treatment over a short duration will allow us to rapidly determine whether sustained treatment with ACZ can alter cerebral blood flow and will provide a go/no-go for future studies using this drug/paradigm. In this stage, all subjects will receive baseline pCASL and DSC MRI, will start oral ACZ continued for 12 consecutive weeks and undergo repeat imaging at predetermined intervals. MRI scans and clinical evaluations will be obtained every 6 weeks with contrast-based scans repeated at 12 weeks. As previously stated, DSC provides measurements of not only CBF but also blood volume (CBV) and transit time of blood (MTT). It is possible that in addition to changes in blood flow, the amount or rate of flow is altered with ACZ and may be an important mechanism to tissue repair. DSC techniques will provide information that cannot be obtained by using pCASL alone that might be important to determine how blood flow is altered with ACZ.

In addition, we will use advanced MRI techniques to also quantify tissue oxygen extraction before and after ACZ therapy as well as determine the metabolic rate of oxygen consumption in various tissues.



Stage 2b – Tachyphylaxis & Long-term Safety/Tolerability

This stage is designed to determine if daily oral ACZ is tolerated and if tachyphylaxis might occur with long-term use for the specific formulation of oral ACZ that we will utilize. Some authors have reported attenuation of the initial increases in CBF with sustained use or oral ACZ though still showing a persistent increase in cerebral perfusion of between 15% - 20%, when compared to pre-treatment levels (Friberg et al., 1990). In this stage, all subjects will receive baseline pCASL and DSC MRI and will start oral ACZ. Patients will continue on 1000 mg BID dose for 24 consecutive weeks and undergo repeat imaging at predetermined intervals. MRI scans and clinical evaluations will be obtained every 6 weeks with contrast-based scans repeated every 12 weeks. Contrast will be administered every 12 weeks to determine DSC measures of blood flow and whether new lesions have formed. As previously stated, DSC provides measurements of not only CBF but also blood volume (CBV) and transit time of blood (MTT). DSC techniques will provide information that cannot be obtained by using pCASL alone that might be important to determine how blood flow is altered with ACZ.

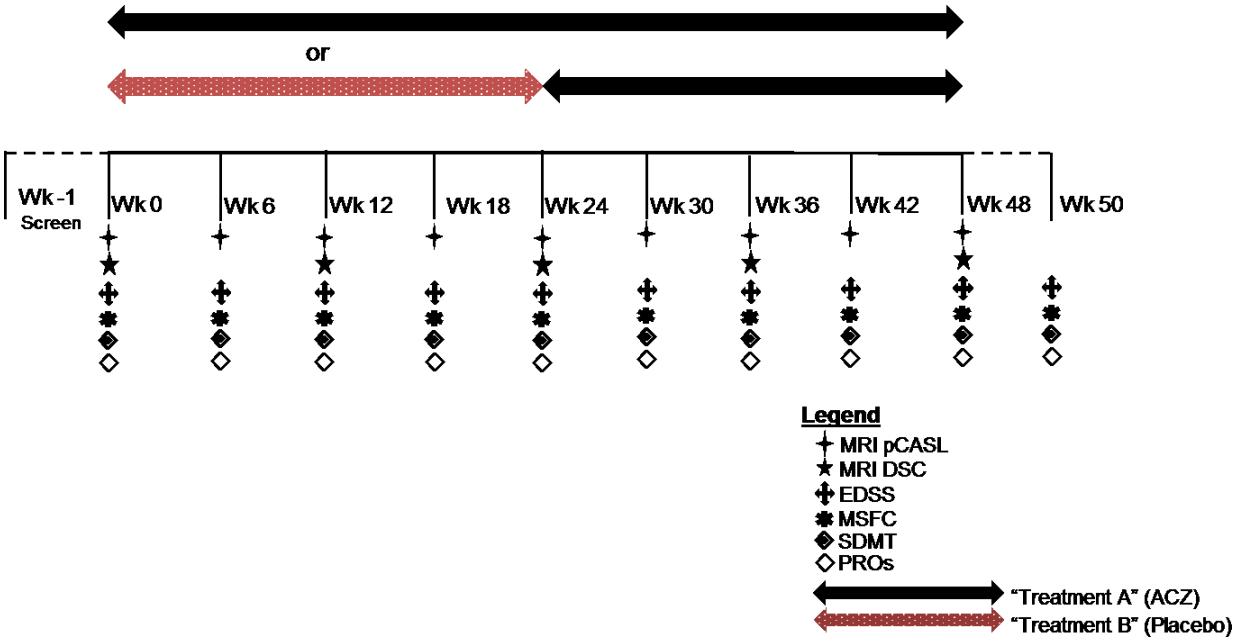
Stage 3 - Many factors contribute to the formation and evolution of lesions in MS (Cotton et al., 2003; Meier and Guttmann, 2006; Narayana et al., 1998). As much as 60% of MS lesions resolve over six

months while the remainder show evidence of persistent damage (Sahraian et al., 2010). In this stage, all subjects will receive baseline pCASL and DSC MRI and will then be randomized to first receive either daily oral therapy with either **"Treatment A",** extended-release ACZ capsules or **"Treatment B"**, placebo capsules for 24 consecutive weeks and undergo repeat imaging at predetermined intervals. MRI scans and clinical evaluations will be obtained every 6 weeks with contrast-based scans repeated every 12 weeks. Contrast will be administered every 12 weeks to determine DSC measures of blood flow and whether new lesions have formed and determine how blood flow is altered with ACZ.

At the end of week 24, patients initially randomized to Treatment B will take 1000 mg extended-release ACZ capsules. Similar imaging and clinical evaluations will be performed for the second 24-week period. All subjects will discontinue drug at week 48 and obtain a follow-up clinical visit two weeks later. Based upon established pharmacokinetics, the half-life of drug is between 60-75 hours and one would expect insignificant levels within two weeks of discontinuation.

To maintain blinding, patients, evaluating clinicians and MRI analysts will not be informed of treatment assignment or sequence of scans.

We anticipate that sustained increases in regional cerebral perfusion over 6 months will result in improved tissue integrity as measured using diffusion tensor metrics and fewer MS lesions that evolve to persistent black holes.



HYPOTHESIS AND OBJECTIVES

Hypothesis

The underlying hypothesis of this proposal is that long-term therapy with ACZ will improve cerebral perfusion in subjects with MS and this improved perfusion will result in improved tissue integrity in a proportion of MS lesions. To test this hypothesis, subjects will be treated with ACZ, a currently FDA approved agent previously shown in healthy controls to increase cerebral perfusion (Sullivan et al., 1987; Vagal et al., 2009). Subjects will be scanned at various time points (see sections 4 and 6.3) and quantitative MRI metrics will be used to evaluate lesion evolution during and after discontinuation

of treatment.

Primary & Key Secondary Objective

Determine the degree to which divided daily doses of ACZ improves global and regional cerebral perfusion in subjects with MS.
Study subjects will obtain serial MRI scans prior to, during and after therapy (see sections 4 and 6.3). Cerebral blood flow will be measured using an optimized pCASL technique as described by Aslan (Aslan et al., 2010). This will allow us to test the first component of our hypothesis that treatment with ACZ will result in increased regional and/or global perfusion.

The primary endpoint of this study will be to determine if therapy with oral divided-dose ACZ administered each day for week-long period results in at least 15% increase in CBF. Studies have shown between 10% - 15% decrease in cerebral perfusion in patients with relapsing MS (Adhya et al., 2006). Therefore, increases in CBF of at least 15% might result in change to lesion evolution. Baseline CBF measures will be compared to measures obtained at each dose escalation step using paired *t* test and Mann-Whitney test.

Determine the effect of long-term oral ACZ on lesion evolution. The key secondary endpoint of this study is to determine if long-term use of oral daily divided-dose with ACZ improves tissue integrity in a proportion of MS lesions.

MRI scans will be segmented using into white and gray matter, T2 and T1 lesion components and contrast-enhancing lesion component. Expert raters (I.V., S.D., L.F.), who are blinded to treatment arm (see section 6.1) will perform quality control of the automated segmentation and obtain quantitative measures of cerebral perfusion. The number and volume of T2 hyperintense and T1 hypointense lesion will be determined using MRIAP. The ratio of T1 hypointense/T2 hyperintense lesion will be determined for baseline and each crossover period and correlated with CBF, CBV and MTT measures obtained at similar times using the Spearman rank correlation.

Diffusion tensor imaging (DTI) is a reliable, validated technique that quantitatively measures molecular diffusivity within tissue and provides information about its microstructural integrity. DTI will be used to monitor tissue integrity and correlate change with increases in CBF following ACZ.

Tertiary Objectives

Several studies have quantitated transverse and longitudinal relaxation times within lesions and normal-appearing white matter and gray matter. These studies have shown that changes in both transverse and longitudinal relaxation can precede lesion formation and possibly better evaluate lesion evolution as compared to black hole volumes (Papadopoulos et al., 2010; van Walderveen et al., 1999a; Walsh et al., 2014; Warntjes et al., 2013). We will manually delineate regions of interest within lesions to obtain T2 and T1 relaxometry metrics. Lesions within areas with greatest change in CBF will be compared with those with least change. We anticipate a correlation between relaxometry measures and areas with varying CBF.

This study will include analysis to determine if therapy with oral divided-dose ACZ impacts global measures of clinical disability. Change in clinical disability measures assessed using the multiple sclerosis functional composite scale (MSFC) and the expanded disability status scale (EDSS) will be correlated with CBF, CBV and MTT. In addition, patient reported clinical outcome measures will be assessed at each time point. As this trial uses a small sample size, we would not expect statistically significant treatment effects on clinical disability though this exploratory measure should be useful in power calculation for the anticipated larger, randomized clinical trial.

Potential Risks and Benefits:

End Points - Efficacy

Altered cerebral perfusion has been previously reported in patients with MS. Global, regional and lesional changes in perfusion have been documented and some have found associations between decreased regional CBF and T2 lesion volume (Ota et al., 2013).
There are several currently approved treatments for MS that, in the aggregate, decrease the potential for formation of new MRI lesions. However, none have been clearly shown to impact the evolution of lesions, once formed. Several studies have shown that clinical disability correlates better with tissue integrity within black holes (Parry et al., 2002; Truyen et al., 1996; van Walderveen et al., 1999b).
ACZ is a generally well tolerated therapy that has previously been shown to transiently increase cerebral perfusion. Long-term use of ACZ might more persistently improve global or regional CBF and could increase the proportion of lesions with improved tissue integrity.

End Points - Safety

ACZ is generally well tolerated with few reported adverse events (AEs). Transient perioral numbness has been reported in patients receiving bolus of IV ACZ. Hypersensitivity reactions, including diffuse pruritus, hives and shortness of breath have been reported with repeat administration of either IV or oral medication. A complete list of AEs is available in the package insert (see section 7) and will be provided to subjects with the informed consent.

Subjects will have face-to-face clinical evaluations at each scheduled visit (see section 4), will have monthly telephone or email contacts during the course of study and encouraged to report any AE occurring with drug at any point during the study. Any subject who is unable to tolerate study medication will be withdrawn from the study.

During the dose escalation, in addition to issues of tolerability, subjects will be monitored at each transition point for the following specific serious AEs: paraesthesia, change in taste, accelerated change in blood pressure or fluid retention. Any subject with serious AEs will be withdrawn from the study. Patients with persistent serious AEs will be treated using standard medical practice.

STUDY POPULATION

List the number of subjects to be enrolled.

This exploratory study is designed to evaluate the long-term effects of oral ACZ on CBF in adult subjects with MS. We will enroll 100 MS subjects between the age of 18 and 55 without history of cerebrovascular disease or glaucoma.

There are several studies suggesting that MS lesions in the pediatric population differ from that typically seen in the adult population (Ghassemi et al., 2014; Renoux et al., 2007). To minimize potential confounding variables, all stages of the study will recruit only adult subjects with MS.

All subjects will be recruited from patients seen at the MS clinic located at the University of Texas Professional building (UTP) Neurology practice[redacted].
Information about the study will be available on the UT Neurology web site and in print format at the UTP MS clinic.

Informed consent will be obtained from each participant prior to entry and, for subjects who elect to

participate in multiple parts of the study, prior to each stage.

Criteria for Recruitment

Subjects who wish to participate in either stage of the study will be evaluated by the principle investigator to determine if they meet inclusion and exclusion criteria. A thorough medical history will be performed to determine if any pre-existing cardiovascular disease or glaucoma is present. Subjects whose history is unknown will be encouraged to obtain evaluation by a primary care physician and eye-care specialist prior to recruitment. In addition, available blood tests, performed within six months of study entry, will be reviewed to determine if any renal or hepatic dysfunction is present.

Potential subjects with historical or laboratory evidence to suggest dysfunction in one of the above clinical systems will be excluded from study participation. As the rate of these comorbidities is low in a young population, we do not anticipate large numbers of failures at this stage of screening.

Inclusion Criteria

Patients who meet all of the following inclusion criteria during the screening period will be eligible for enrolment in this study:

1. Age between 18 and 55 years, inclusive.
2. Diagnosis of relapsing forms of multiple sclerosis using revised McDonald criteria (Polman et al., 2011).
3. Stable on any FDA-approved disease modifying therapy (DMT). The term “stable” implies that the subject has not had change in therapy for any reason for the six months prior to study entry.
4.
5. EDSS 0 – 6.0 inclusive (Kurtzke, 1983).
6. Understood and signed written informed consent, obtained prior to the study subject undergoing any study-related procedure, including screening tests.

Exclusion Criteria

Patients who meet any of the following exclusion criteria during the screening period will not be eligible for enrollment in this study:

1. Known hypersensitivity to sulfonamides or derivatives
2. Known history of renal or hepatic disease, cerebrovascular disease including stroke, transient ischemic attack, myocardial infarction, angina or congestive heart failure
3. Evidence to suggest hyponatremia or hypokalemia, marked kidney dysfunction defined as creatinine greater than 2.0 mg/dL or liver disease dysfunction defined as aspartate aminotransferase (AST) or alanine aminotransferase (ALT) greater than three-fold upper limit of normal (ULN).
4. Evidence to suggest suprarenal gland failure.
5. Evidence of hyperchloremic acidosis.
6. Initiation of new immunosuppressant treatment after the subject becomes protocol-eligible (except for corticosteroids) or enrolment in a concurrent trial.
7. Prior treatment with mitoxantrone, natalizumab, rituximab, methotrexate, cladribine, cyclophosphamide or within 6 months of initiation of study.
8. Subjects with any history of cytopenia.
9. History of pulmonary obstruction or emphysema.
10. Active hepatitis B or hepatitis C infection or evidence of cirrhosis.

11. Human immunodeficiency virus (HIV) positivity.
12. Uncontrolled diabetes mellitus defined as HbA1c > 8% and/or requiring intensive management.
13. Uncontrolled viral, fungal, or bacterial infection (excluding asymptomatic bacteriuria).
14. Any condition that, in the opinion of the investigators, would jeopardize the ability of the subject to tolerate treatment with ACZ.
15. Prior history of malignancy.
16. Positive pregnancy test or inability or unwillingness to use effective means of birth control. Effective birth control is defined as:
    - Refraining from all acts of vaginal intercourse (abstinence)
    - Consistent use of birth control pills
    - Tubal sterilization or male partner who has undergone vasectomy
    - Placement of an IUD (intrauterine device)
    - Use, with every act of intercourse, of a diaphragm with contraceptive jelly and/or condoms with contraceptive foam
17. Presence of metallic objects implanted in the body that would preclude the ability of the subject to safely have MRI exams.
18. Psychiatric illness, mental deficiency, or cognitive dysfunction making compliance with treatment or informed consent impossible.

Withdrawal Criteria

Patients can be withdrawn at any time if the investigator concludes that it would be in the patients' best interest for any reason. Patients may voluntarily withdraw from the study for any reason at any time. They may be considered withdrawn if they state an intention to withdraw, or fail to return for visits, or become lost to follow up for any other reason. Protocol violations should not lead to patient withdrawal unless they indicate a significant risk to the patient's safety. If premature patient withdrawal occurs for any reason, the principle investigator must determine the primary reason for a patient's premature withdrawal from the study and record this information on the Unanticipated Problems Tracking Log.

For patients who are lost to follow-up (i.e., those patients whose status is unclear because they fail to appear for study visits without stating an intention to withdrawal), the investigator will show "due diligence" by documenting in the source documents steps taken to contact the patient, e.g., dates of telephone calls, registered letters, etc. In the case of death, a patient will be considered withdrawn.

Subject Replacement

Patients who prematurely withdraw will not be replaced.

TRIAL SCHEDULE

Visit Schedule:

Stage 1a – IV ACZ in MS Subjects						
Total of 10 subjects (5 healthy and 5 MS patients)						
	Day -7 Screen	0 min	15 min	30 min	120 min	240 min
Treatment		1 gm IV bolus ACZ administered immediately after '0 min' pCASL scan				
H & P	X					
CBC, CMP	X					
Urine pregnancy	X					
pCASL MRI		X	X	X	X	X
DSC MRI						X

Stage 1b –Oral ACZ dose-escalation in MS Subjects						
Up to a Total of 10 subjects						
	Day -7 Screen	Wk 0 Visit 1	Wk 2 Visit 2	Wk 4 Visit 3	Wk 6 Visit 4	Wk 8 Visit 6
Treatment		500 mg PO BID	1000 mg PO BID	1500 mg PO BID	2000 mg PO BID	
H & P	X	X	X	X	X	X
CBC, CMP	X					X
Urine pregnancy*	X					
pCASL MRI		X	X	X	X	X
MSFC		X	X	X	X	X
EDSS		X	X	X	X	X
Blood Sample		X	X	X	X	X
* Female participants who have already been evaluated for pregnancy will not be retested unless otherwise indicated						

•												

Stage 2a – Safety/Tolerability & Kinetics of Treatment Effect					
• Up to a Total of 10 subjects					
	Wk -4 Screen	Wk 0 Visit 1	Wk 6 Visit 2	Wk 12 Visit 3	Wk 14 Visit 4
Treatment		1000 mg ACZ BID		d/c ACZ	
H & P	X	X	X	X	X
CBC, CMP		X	X	X	X
Urine pregnancy*		X			
DSC MRI		X		X	
pCASL MRI		X	X	X	X
MSFC		X	X	X	X
EDSS		X	X	X	X
MFIS, MSWS-12		X	X	X	X
Blood Sample		X	X	X	X
* Female participants who have already been evaluated for pregnancy will not be retested unless otherwise indicated					

- Additional CBC,CMP will be performed if measured ALT/AST > 1X ULN or if other alterations above baseline
- EDSS – Expanded disability status scale
- MSFC – Multiple Sclerosis Functional Composite Scale

Stage 2b – Tolerability & Tachyphylaxis of ACZ in MS Subjects						
• Up to a Total of 10 subjects						
	Wk -1 Screen	Wk 0 Visit 1	Wk 6 Visit 2	Wk 12 Visit 3	Wk 18 Visit 4	Wk 24 Visit 5
Treatment		ACZ at dose identified in Stage 1b				
H & P	X	X	X	X	X	X
CBC, CMP		X	X	X	X	X
Urine pregnancy*		X				
DSC MRI		X		X		X
pCASL MRI		X	X	X	X	X
MSFC		X	X	X	X	X
EDSS		X	X	X	X	X
MFIS, MSWS-12		X	X	X	X	X
Blood Sample		X	X	X	X	X
* Female participants who have already been evaluated for pregnancy will not be retested unless otherwise indicated						

- Additional CBC,CMP will be performed if measured ALT/AST > 1X ULN or if other alterations above baseline
- EDSS – Expanded disability status scale
- MSFC – Multiple Sclerosis Functional Composite Scale

Stage 3 – RCT; Long-term therapy with ACZ in MS Subjects											
• Delayed-start design with oral therapy **"Treatment A"** (ACZ) or **"Treatment B"** (placebo) • Total of 60 subjects											
	Wk -1 Screen	Wk 0 Visit 1	Wk 6 Visit 2	Wk 12 Visit 3	Wk 18 Visit 4	Wk 24 Visit 5	Wk 30 Visit 7	Wk 36 Visit 8	Wk 42 Visit 9	Wk 48 Visit 10	Wk 50 Visit 11
Treatment		Randomize to ACZ or placebo								d/c treatment	End Study
H & P	X	X	X	X	X	X	X	X	X	X	
CBC, CMP		X	X	X	X	X	X	X	X	X	
Urine pregnancy*		X									
DSC MRI		X		X		X		X		X	
pCASL MRI		X	X	X	X	X	X	X	X	X	X
MSFC		X	X	X	X	X	X	X	X	X	X
EDSS		X	X	X	X	X	X	X	X	X	X
MFIS, MSWS-12		X	X	X	X	X	X	X	X	X	X
Blood Sample		X	X	X	X	X	X	X	X	X	X
* Female participants who have already been evaluated for pregnancy will not be retested unless otherwise indicated											

- Additional CBC,CMP will be performed if measured ALT/AST > 1X ULN or if other alterations above baseline
- EDSS – Expanded disability status scale
- MSFC – Multiple Sclerosis Functional Composite Scale

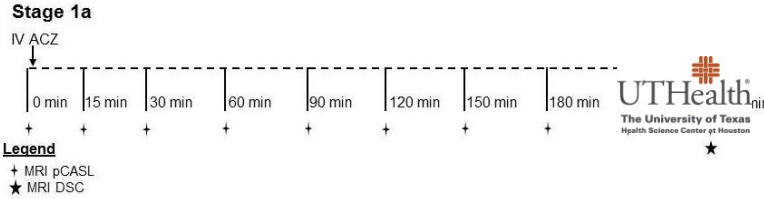
STUDY DESIGN

Summary of Study Design

The initial stages of this study are designed as a proof of principle to show that ACZ therapy improves cerebral perfusion in MS subjects. The final stage of this study is a prospective crossover design to evaluate the potential for ACZ to persistently enhance cerebral perfusion and impact the evolution of T2 hyperintense MS lesions into permanent black holes. This pilot study is anticipated to lead to a larger randomized phase 3 clinical trial.

Participants will be recruited from the MS patient population seen at the UTP practice. The MS Clinic is a National MS Society affiliated comprehensive and coordinated center for MS care and a member of the Consortium of MS Centers. This clinic evaluates approximately 2700 MS patients per year. Given the safety and tolerability of study drug, we anticipate recruitment of two MS subjects each month for all stages of this study.

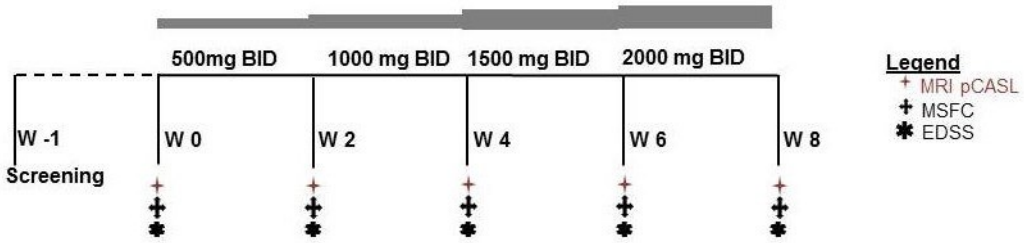
Stage 1a - This stage will determine the degree of change, temporal kinetics and difference between various regions in cerebral perfusion after IV ACZ in subjects with MS.



In total, this portion of the study is anticipated to be complete within 2.5 months. ACZ therapy and all image acquisition will be completed on the same day for subjects enrolled in stage 1a.

As all subjects will be treated with IV ACZ, the image analyst will be unblinded during this stage. Cerebral perfusion measures will be determined by S.D.

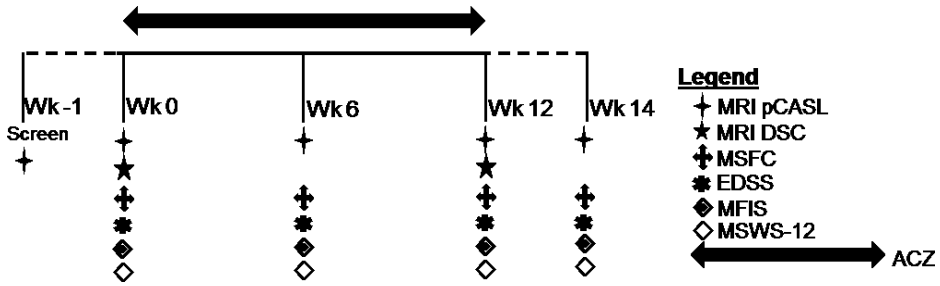
Stage 1b - Participants in this stage of the study will be given escalating divided doses of oral ACZ up to 4000 mg per day to determine both the magnitude of change to cerebral perfusion with maintenance daily oral ACZ therapy and to define the optimum dose(s) for additional studies. Stage 1b and 2 of the study will utilize extended release oral ACZ, distributed by Hermann Research pharmacy and self-administered by the subject.



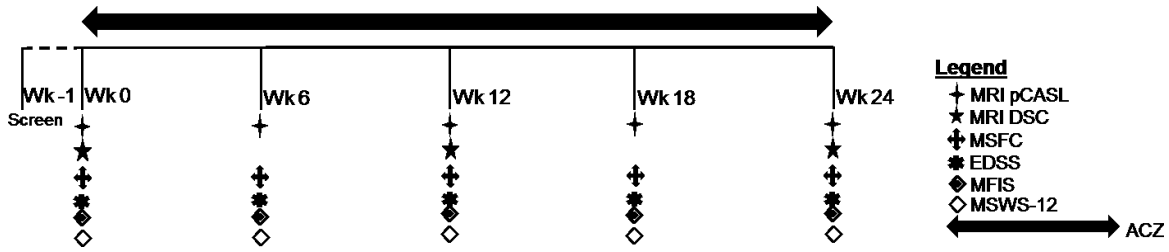
Treatment and data collection for stage 1b will take 2.5 months for each enrolled subject. As many as two subjects can be evaluated during the month and we would anticipate that this portion of the study can be completed within twelve months.

Image analysis will be performed once each subject completes treatment. Cerebral perfusion measures will be determined for each dose by the same unblinded analyst (S.D.).

Stage 2a - This stage will continue to gather information about whether daily oral ACZ is tolerated and the kinetics of the treatment effect. Patients continue on the same dose for 12 consecutive weeks and undergo repeat imaging at predetermined intervals. Sustained treatment over a short duration will allow us to rapidly determine whether sustained treatment with ACZ can alter cerebral blood flow and will provide a go/no-go for future studies using this drug/paradigm. Patients will obtain MRI scans and clinical evaluations every 6 weeks with contrast-based scans repeated at 12 weeks.



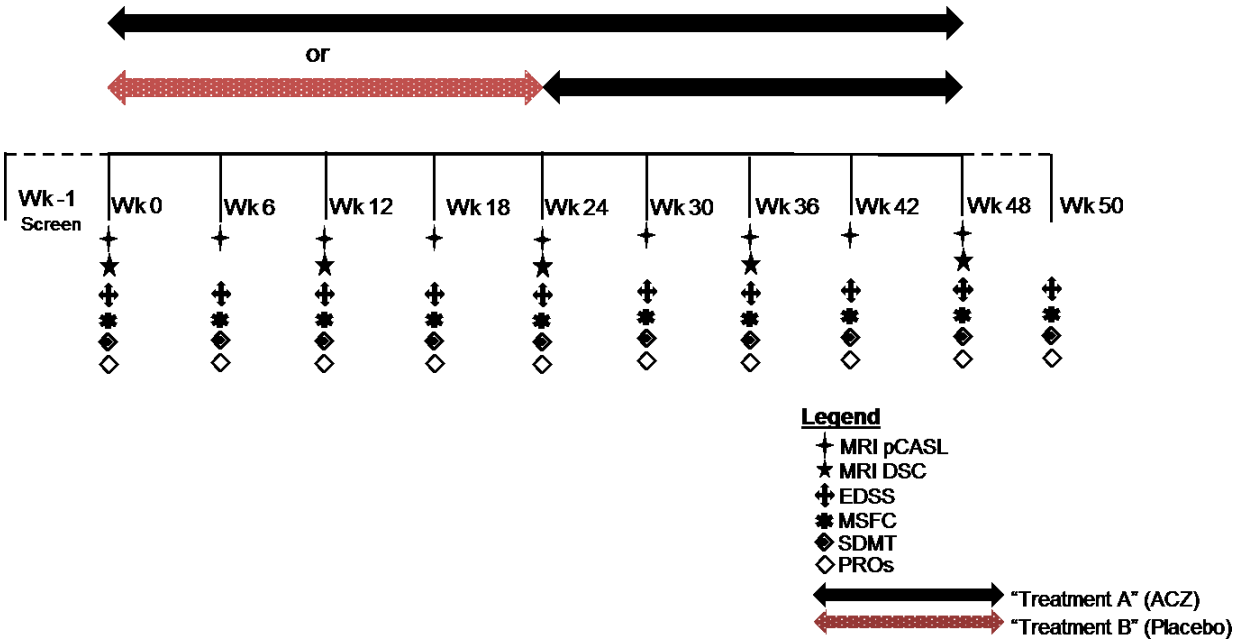
Stage 2b - This stage will determine long-term tolerability and if tachyphylaxis is present with oral ACZ. Patients will continue on the same dose for 24 consecutive weeks and undergo repeat imaging at predetermined intervals. MRI scans and clinical evaluations will be obtained every 6



weeks with contrast-based scans repeated every 12 weeks.

Stage 3 - This stage is a prospective delayed-start design to evaluate the potential for ACZ to persistently enhance cerebral perfusion and impact the evolution of MS lesions. This pilot study is anticipated to lead to a larger randomized phase 3 clinical trial.

Subjects will obtain both contrast-enhanced and non-contrast MRI to fully evaluate both existing and newly formed MS lesions. In addition, contrast based analysis of cerebral perfusion offers measurements of CBV and MTT of blood, data not available with pCASL alone. DSC techniques will provide information that cannot be obtained by using pCASL alone that might be important to determine how blood flow is altered with ACZ.



Subjects will be randomized to either ACZ or placebo first. Both subjects and raters (both clinical and imaging) will be blinded to treatment arm. Image analysis will be performed at the end of study. Each subject scan will be assigned a random five-digit number. Experienced imaging analysts (I.V., S.D., L.F.), blinded to treatment arm, will obtain manual and semi-automatic quantitative measures of global and regional cerebral blood flow, T2 lesion volume, T1 lesion volume, T1-gadolinium (Gd) positive lesion volume, gray and white matter volume and global and regional atrophy. We will compare quantitative imaging measures between treatments.

METHODS AND ASSESSMENTS

Patient recruitment, consenting and database procedures – All subjects for the study will be specifically recruited from the Neurology clinic located in the UTP building. Recruitment of all subjects will initially be sequential, based upon planned follow-up visits to the Neurology Clinic. Subsequent adjustments to patient recruitment might be introduced based upon periodic evaluation of evolving data that might dictate a more focused approach within certain patient groups. We anticipate that patient volunteers can be identified and consented on the day of their regularly scheduled Neurology Clinic visit. Thus, the first level of potential bias in the subjects studied will simply be that of the stochastic nature of the scheduled visits. However, there may be additional selection characteristics placed on recruitment of patient volunteers for the studies, should age or gender characteristics of the MS patients not be well reflected in the accumulating group.

As patients consent for the study they will be given a unique patient study code number that will be used to identify them in a database specifically constructed for the study. Primary consent will concentrate on the nature of the study, a description of the database and their consent to be re-contacted by study personnel for future participation in the study. The primary consent will include a brief description of advanced brain MRI techniques that will be utilized. Any patient contacted later for their interest in other parts of this trial will then be re-consented, with the next procedure and its potential risks described in appropriate detail. All necessary patient demographics will be collected for entry into the database by a study coordinator/data entry manager (James Jemelka).

Data handling and storage – The MRI-Analysis Center (MRI-AC), previously directed by Dr. Wolinsky and currently directed by me, has extensive experience with the management of large amounts of deidentified patient related data and images gathered for both local research studies and national

and international studies on thousands of subjects, each with multiple sessions. All data is stored on the MRI-AC macpdbs1 server physically secured in the University Professional Tower Data Center located within virtual Zone 100 (contains servers with protected health information as well as other sensitive or critical data; no access may be initiated directly from the Internet to Zone 100; all servers in Zone 100 are geographically located in secure areas). All workstations physically located in the MRI-AC are confined to Zone 40 (contains the bulk of the computing resources geographically distributed throughout the UTHSCH campus; no access may be initiated directly from the Internet into Zone 40; protected health information must be encrypted; rules for traffic initiated form Zone 40 to other areas: Internet is allowed, Zone 20 is allowed, Zone 100 is restricted to defined servers and protocols). Only individuals who can authenticate to the VPN appliance connected to Zone 40 and have appropriate authorization privileges may access MRI-AC resources from the Internet. Port configurations of the firewalls separating the Zones are managed by the UTHSCH Information Security Staff. Port configuration is managed by a firewall database in accordance with firewall policies and procedures. The MRI-AC Director is the information owner of the macpdbs1 server and the workstations. The steward of the macpdbs1 server is the Data Center Operations and Services (DCOS) division of the UTHSCH Information Technology Department. The steward of the MRI-AC workstations is the Medical School Information Technology (MSIT) Office. We will utilize these resources to manage and store all data and digital images generated as part of this project.

Randomization and Blinding

During stage 3 of this study, subjects will be randomized to either first receive ACZ or placebo. Both subjects and raters (clinical and image analysts) will be blinded to treatment arm.

MRI scans for each subject will be assigned a unique non-contiguous random ID using a 5-digit random number generator and all imaging raters (I.V., S.D., L.F.) will be blinded to treatment arm. A semi-automated, previously validated tissue segmentation software (FreeSurfer v5.3, https://surfer.nmr.mgh.harvard.edu/) will be used to determine T2 hyperintense (lesion) and T1 hypointense (black hole) volumes. A separate expert rater (K.H.) will review and analyse DTI images. An expert rater (I.V), blinded both to treatment arm and sequence of scan acquisition, will perform quality control checks of software segmentation.

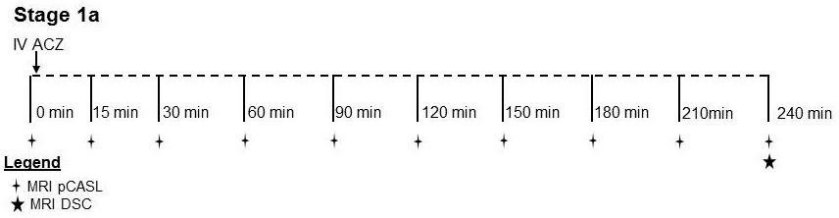
Image analysis to determine regional and global CBF as well as atrophy will be performed by additional qualified expert raters (S.D., L.F., K.H.) who are blinded to the treatment arm.

Contraception and Pregnancy Testing

Female participants must use effective means of birth control for the duration of the study. Effective birth control is defined as refraining from all acts of vaginal intercourse (abstinence), consistent use of birth control pills, tubal sterilization or male partner who has undergone vasectomy, placement of an IUD (intrauterine device), or use of a diaphragm with contraceptive jelly and/or condoms with contraceptive foam with every act of intercourse.
Study participants will receive urine pregnancy tests prior to ACZ and as scheduled in the trial schedule (see section 4).

Study Visits and Procedures

Stage 1a:



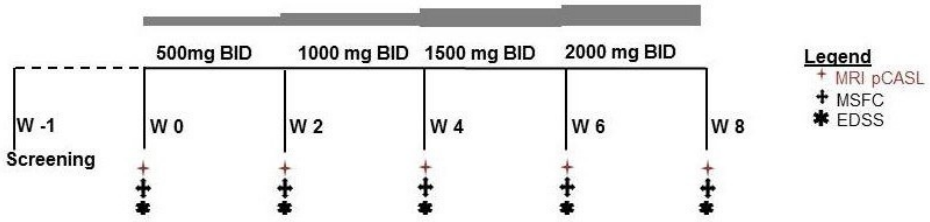
Screening Visit – Subjects will receive a complete medical and neurological examination. In addition, all subjects will obtain CBC, CMP and, if female, urine pregnancy test.

Subjects will receive non-contrast MRI brain with scout, 3DT1 and pCASL sequences immediately prior to infusion of IV ACZ. The scout sequence is used to establish proper subject orientation and slice prescription and requires less than 5 minutes. The 3DT1 is a rapid sequence that allows for proper registration between scans and will be obtained after the scout images and again if the patient is removed from the MRI gantry. Finally, pCASL sequences will be performed to determine CBF. The group of sequences including pCASL will collectively require about 12 minutes.

Subjects will receive 1 gram bolus of IV ACZ and a second scan using pCASL sequences will be obtained within 15 minutes of infusion. Subjects will remain in the MRI gantry and receive a third scan to determine CBF at 30 minutes from time of infusion. Subjects will then be removed from the MRI gantry and allowed a break.

Subjects will again be placed on the MRI gantry and scout, 3DT1 and pCASL sequences obtained within 120 minutes from time of ACZ infusion. Subjects will then be removed from the MRI gantry and allowed a break. A final scan will be performed using the same sequences within 240 minutes from time of ACZ infusion.

Stage 1b:



Screening Visit – Subjects will receive a complete medical and neurological examination. In addition, all subjects will obtain CBC, CMP and, if female, urine pregnancy test.

Visit 1 – Subjects will receive non-contrast MRI brain using sequences previously described to ensure proper orientation and registration (scout, 3DT1), sequences necessary for segmentation using MRIAP (2D dual echo, 2D fluid-attenuated inversion recovery [FLAIR]) and pCASL sequences to determine CBF at baseline. Subjects will begin taking 250 mg oral ACZ twice daily and continue for two weeks. Subjects will be encouraged to call the PI with any adverse events (see sections 2.4b and 7.1.2) while on study drug.

Visit 2 – Prior to dose-escalation, subjects will receive non-contrast MRI brain sequences as described above to measure effect of previous oral daily divided dose of ACZ on CBF. Upon completion of MRI, subjects will begin taking 500 mg oral ACZ twice daily and continue for two weeks. Subjects will be encouraged to call the PI with any adverse events (see sections 2.4b and 7.1.2) while on study drug.

Visit 3 – Prior to dose-escalation, subjects will receive non-contrast MRI brain sequences as described above to measure effect of previous oral daily divided dose of ACZ on CBF. Upon completion of MRI, subjects will begin taking 1000 mg oral ACZ twice daily and continue for two weeks. Subjects will be encouraged to call the PI with any adverse events (see sections 2.4b and 7.1.2) while on study drug.

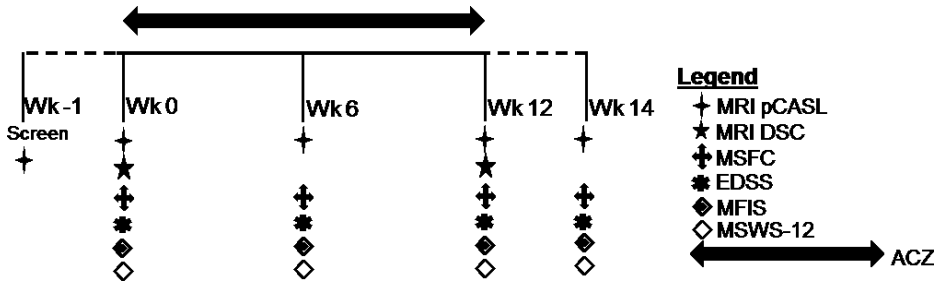
Visit 4 – Prior to dose-escalation, subjects will receive non-contrast MRI brain sequences as described above to measure effect of previous oral daily divided dose of ACZ on CBF. Upon completion of MRI, subjects will begin taking 1500 mg oral ACZ twice daily and continue for two weeks. Subjects will be encouraged to call the PI with any adverse events (see sections 2.4b and 7.1.2) while on study drug.

Visit 5 – Prior to dose-escalation, subjects will receive non-contrast MRI brain sequences as described above to measure effect of previous oral daily divided dose of ACZ on CBF. Upon completion of MRI, subjects will begin taking 2000 mg oral ACZ twice daily and continue for two weeks. Subjects will be encouraged to call the PI with any adverse events (see sections 2.4b and 7.1.2) while on study drug.

Visit 6 – Subjects will receive non-contrast MRI brain sequences as described above to measure effect of previous oral daily divided dose of ACZ on CBF. Upon completion of MRI, subjects will discontinue use of ACZ.

Stage 2a:

c)



Screening Visit – Subjects will receive a complete medical and neurological examination and obtain screening EDSS evaluation. In addition, all subjects will obtain CBC, CMP and, if female, urine pregnancy test.

Visit 1 – Subjects will receive MRI brain with and without gadolinium contrast utilizing the following sequences in the order listed:

Scout and 3D T1-weighted images to ensure proper orientation and registration
2D dual echo, 3D FLAIR necessary for segmentation
Muti-shell DWI (DTI sequences)
A single shot 2D echo-planar imaging (EPI) for pCASL-based measures of CBF
2D T1-weighted images prior to Gd administration
DSC sequence for measures of CBF, CBV, MTT
2D T1-weighted images after Gd administration

In addition to segmenting MS lesion (T2 hyperintense), we are able to segment T1 pre- and post-Gd lesion volumes. Images obtained at the site at UT are routinely subject to review by a radiologist who is not further involved in image analysis.

Visit 2 – Subjects will receive a complete medical and neurological examination, MSFC & EDSS evaluation as well as patient-reported clinical disability measures (MFIS & MSWS-12) and will be asked questions to ascertain tolerability to daily therapy. All subjects will obtain MRI brain without gadolinium contrast utilizing the sequences described above except for DSC sequence.

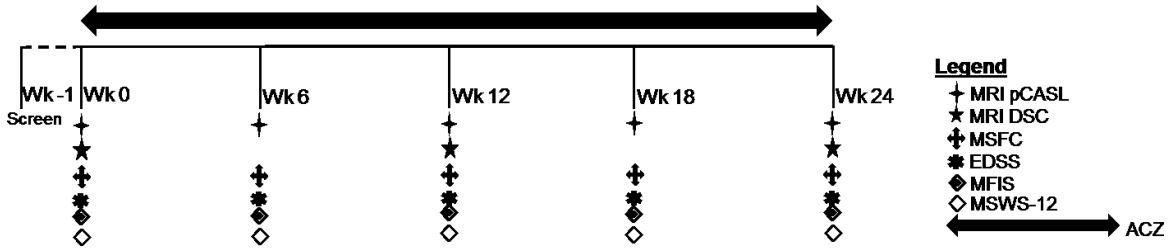
Visit 3 – Subjects will receive a complete medical and neurological examination, MSFC & EDSS evaluation as well as patient-reported clinical disability measures (MFIS & MSWS-12) and will be asked questions to ascertain tolerability to daily therapy. All subjects will obtain MRI brain with and without gadolinium contrast utilizing the sequences described above.

Upon completion of the MRI scan, all subjects will be instructed to discontinue ACZ treatment.

Visit 4 – Subjects will receive a complete medical and neurological examination, MSFC & EDSS evaluation as well as patient-reported clinical disability measures (MFIS & MSWS-12) and will be asked questions to ascertain tolerability to daily therapy. All subjects will obtain MRI brain without gadolinium contrast utilizing the sequences described above except for DSC sequence.

a.

Stage 2b:



Screening Visit – Subjects will receive a complete medical and neurological examination and obtain screening EDSS evaluation. In addition, all subjects will obtain CBC, CMP and, if female, urine pregnancy test.

Visit 1 – Subjects will receive MRI brain with and without gadolinium contrast utilizing the MRI sequences as detailed in Stage 2a.

An MRI scan with the sequences listed above as well as an MSFC & EDSS evaluation by a blinded rater (L.F.) will be obtained prior to treatment to determine baseline values. Patients will also complete subjective disability outcome measures MFIS and MSWS-12. Upon completion of the baseline scan, all subjects will start ACZ therapy and will continue for 24 weeks.

Subjects will be contacted by telephone or email monthly to document tolerability and encouraged to report any AE occurring with drug at any point during the study.

Visit 2 – Subjects will receive a complete medical and neurological examination, MSFC & EDSS evaluation as well as patient-reported clinical disability measures (MFIS & MSWS-12) and will be asked questions to ascertain tolerability to daily therapy. All subjects will obtain MRI brain without gadolinium contrast utilizing the sequences described above except for DSC sequence.

Visit 3 – Subjects will receive a complete medical and neurological examination, MSFC & EDSS evaluation as well as patient-reported clinical disability measures (MFIS & MSWS-12) and will be asked questions to ascertain tolerability to daily therapy. All subjects will obtain MRI brain with and without gadolinium contrast utilizing the sequences described above.

Visit 4 – Subjects will receive a complete medical and neurological examination, MSFC & EDSS evaluation as well as patient-reported clinical disability measures (MFIS & MSWS-12) and will be asked questions to ascertain tolerability to daily therapy. All subjects will obtain MRI brain without gadolinium contrast utilizing the sequences described above except for DSC sequence.

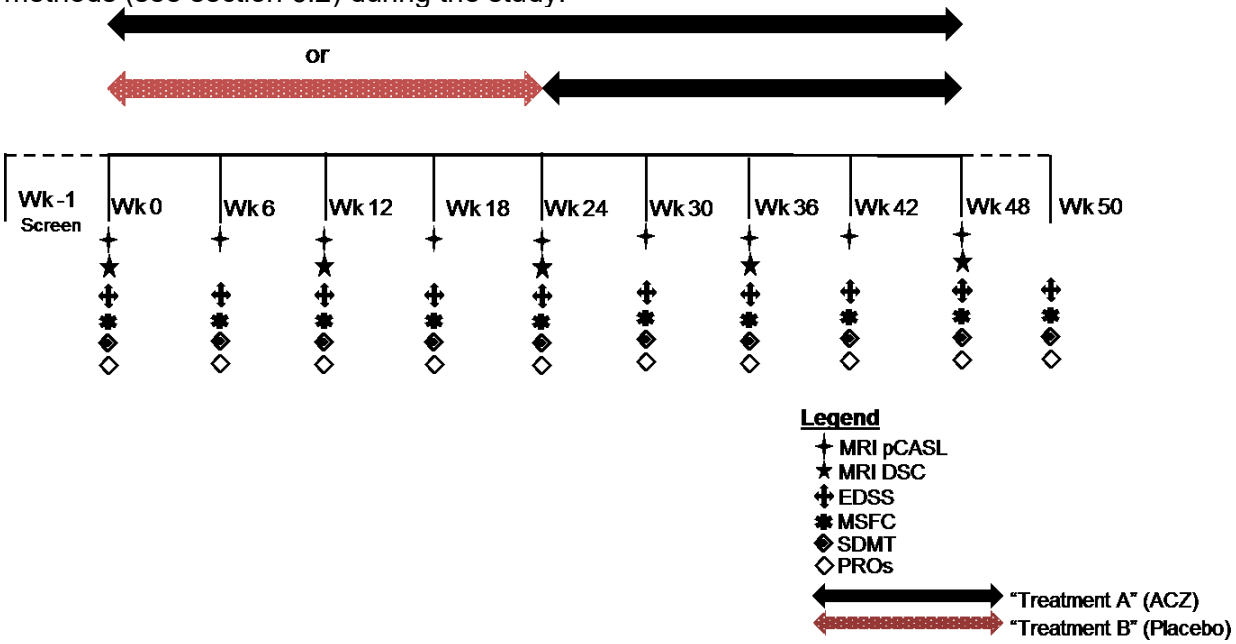
Visit 5 – Subjects will receive a complete medical and neurological examination, MSFC & EDSS evaluation as well as patient-reported clinical disability measures (MFIS & MSWS-12) and will be asked questions to ascertain tolerability to daily therapy. All subjects will obtain MRI brain with and without gadolinium contrast utilizing the sequences described above.

Upon completion of the MRI scan, all subjects will be instructed to discontinue treatment.

Stage 3: Screening Visits and Procedures

The screening visit will occur within one week of study entry. Subjects with relapsing forms of multiple sclerosis without evidence of other neurologic or cardiovascular disease will be recruited for this study.

Subjects will provide a complete medical history and undergo a complete medical and neurological exam. Blood tests (complete blood counts [CBC] and complete metabolic panel [CMP]) will be obtained from every study subject. Female subjects will also be given a urine pregnancy test during the screening visit. In addition, all subjects will undergo a screening MSFC and EDSS (Kurtzke, 1983).
All subjects will be required to discontinue use of herbal supplements for the duration of the study. Finally, both male and female subjects will be counselled on the use of contraceptive methods (see section 6.2) during the study.



Screening Visit – Subjects will receive a complete medical and neurological examination and obtain screening EDSS evaluation. In addition, all subjects will obtain CBC, CMP and, if female, urine pregnancy test.

Visit 1 – Subjects will be randomized to receive Treatment A or Treatment B first for the initial 24 weeks. Subjects will receive MRI brain with and without gadolinium contrast utilizing MRI sequences as detailed in Stage 2a.

Subjects will be contacted by telephone or email monthly to document tolerability and encouraged to report any AE occurring with drug at any point during the study.

Visit 2 – Subjects will receive a complete medical and neurological examination, MSFC & EDSS evaluation as well as patient-reported clinical disability measures (MFIS & MSWS-12) and will be asked questions to ascertain tolerability to daily therapy. All subjects will obtain MRI brain without gadolinium contrast utilizing the sequences described above except for DSC sequence.

Visit 3 – Subjects will receive a complete medical and neurological examination, MSFC & EDSS evaluation as well as patient-reported clinical disability measures (MFIS & MSWS-12) and will be asked questions to ascertain tolerability to daily therapy. All subjects will obtain MRI brain with and without gadolinium contrast utilizing the sequences described above.

Visit 4 – Subjects will receive a complete medical and neurological examination, MSFC & EDSS evaluation as well as patient-reported clinical disability measures (MFIS & MSWS-12) and will be asked questions to ascertain tolerability to daily therapy. All subjects will obtain MRI brain

without gadolinium contrast utilizing the sequences described above except for DSC sequence.

Visit 5 – Subjects will receive a complete medical and neurological examination, MSFC & EDSS evaluation as well as patient-reported clinical disability measures (MFIS & MSWS-12) and will be asked questions to ascertain tolerability to daily therapy. All subjects will obtain MRI brain with and without gadolinium contrast utilizing the sequences described above.

Upon completion of the MRI scan, subjects previously treated with Treatment B will start Treatment A and all subjects will continue on Treatment A for the remaining 24 weeks of the study.

Visit 6 – Subjects will receive a complete medical and neurological examination, MSFC & EDSS evaluation as well as patient-reported clinical disability measures (MFIS & MSWS-12) and will be asked questions to ascertain tolerability to daily therapy. All subjects will obtain MRI brain with and without gadolinium contrast utilizing the sequences described above.

Visit 7 – Subjects will receive a complete medical and neurological examination, MSFC & EDSS evaluation as well as patient-reported clinical disability measures (MFIS & MSWS-12) and will be asked questions to ascertain tolerability to daily therapy. All subjects will obtain MRI brain without gadolinium contrast utilizing the sequences described above except for DSC sequence.

Visit 8 – Subjects will receive a complete medical and neurological examination, MSFC & EDSS evaluation as well as patient-reported clinical disability measures (MFIS & MSWS-12) and will be asked questions to ascertain tolerability to daily therapy. All subjects will obtain MRI brain with and without gadolinium contrast utilizing the sequences described above.

Visit 9 – Subjects will receive a complete medical and neurological examination, MSFC & EDSS evaluation as well as patient-reported clinical disability measures (MFIS & MSWS-12) and will be asked questions to ascertain tolerability to daily therapy. All subjects will obtain MRI brain without gadolinium contrast utilizing the sequences described above except for DSC sequence.

Visit 10 – Subjects will receive a complete medical and neurological examination, MSFC & EDSS evaluation as well as patient-reported clinical disability measures (MFIS & MSWS-12) and will be asked questions to ascertain tolerability to daily therapy. All subjects will obtain MRI brain with and without gadolinium contrast utilizing the sequences described above. Upon completion of the brain MRI, all subjects will be instructed to discontinue assigned treatment.

Visit 11 – Subjects will receive a complete medical and neurological examination, MSFC & EDSS evaluation as well as patient-reported clinical disability measures (MFIS & MSWS-12) and will be asked questions to ascertain any other AEs after discontinuation of therapy. All subjects will obtain MRI brain without gadolinium contrast utilizing the sequences described above except for DSC sequence.

Subjects will be contacted by telephone or email monthly to document tolerability and encouraged to report any AE occurring with drug at any point during the study.

For any part of the study, a subject whose CBC or CMP is not at or near baseline values will repeat these blood tests.

a. Final Study Visit:

The final study visit will be used to document any potential adverse event associated with treatment arm, not previously documented.

b. Post Study Follow-up and Procedures

Subjects will be referred to their primary care physician for continued monitoring as directed should there be persistent adverse events from use of study drug.

c. Discontinuation Visit and Procedures

Subjects may withdraw from study for any reason and will not be replaced. Subjects who choose to withdraw from the study will be encouraged to complete the end-of-study visit.

Patients with intolerable paraesthesia, change in taste, accelerated change in blood pressure or fluid retention will be withdrawn from the study. Patients with persistent AEs will be referred to their primary care physician for continued monitoring and treatment using standard or care practices.

TRIAL MATERIALS

. Drug Substance: Intravenous Acetazolamide

Package Insert for Intravenous Acetazolamide:

AcetaZOLAMIDE for Injection USP

For Intravenous Use.

Rx ONLY.

DESCRIPTION

Acetazolamide, an inhibitor of the enzyme carbonic anhydrase, is a white to faintly yellowish white crystalline, odorless powder, weakly acidic, very slightly soluble in water and slightly soluble in alcohol. The chemical name for acetazolamide is N-(5-Sulfamoyl-1,3,4-thiadiazol-2yl)-acetamide and has the following structural formula: M.W. 222.24 C4H6N4O3S2

Acetazolamide is available for intravenous use, and is supplied as a sterile powder requiring reconstitution. Each vial contains acetazolamide sodium equivalent to 500 mg of acetazolamide. The bulk solution is adjusted to pH 9.6 using sodium hydroxide and, if necessary, hydrochloric acid prior to lyophilization.

CLINICAL PHARMACOLOGY

Acetazolamide is a potent carbonic anhydrase inhibitor, effective in the control of fluid secretion (e.g., some types of glaucoma), in the treatment of certain convulsive disorders (e.g., epilepsy) and in the promotion of diuresis in instances of abnormal fluid retention (e.g., cardiac edema). Acetazolamide is not a mercurial diuretic. Rather, it is a nonbacteriostatic sulfonamide possessing a chemical structure and pharmacological activity distinctly different from the bacteriostatic sulfonamides.

Acetazolamide is an enzyme inhibitor that acts specifically on carbonic anhydrase, the enzyme that catalyzes the reversible reaction involving the hydration of carbon dioxide and the dehydration of carbonic acid. In the eye, this inhibitory action of acetazolamide decreases the secretion of aqueous humor and results in a drop in intraocular pressure, a reaction considered desirable in cases of glaucoma and even in certain nonglaucomatous conditions. Evidence seems to indicate that acetazolamide has utility as an adjuvant in the treatment of certain dysfunctions of the central nervous system (e.g., epilepsy). Inhibition of carbonic anhydrase in this area appears to retard abnormal paroxysmal, excessive discharge from central nervous system neurons. The diuretic effect of acetazolamide is due to its action in the kidney on the reversible reaction involving hydration of carbon dioxide and dehydration of carbonic acid. The result is renal loss of HCO3 ion, which carries out sodium, water, and potassium. Alkalinization of the urine and promotion of diuresis are thus effected. Alteration in

ammonia metabolism occurs due to increased reabsorption of ammonia by the renal tubules as a result of urinary alkalinization.

INDICATIONS AND USAGE

For adjunctive treatment of: edema due to congestive heart failure; drug-induced edema; centrencephalic epilepsies (petit mal, unlocalized seizures); chronic simple (open-angle) glaucoma, secondary glaucoma, and preoperatively in acute angle-closure glaucoma where delay of surgery desired in order to lower intraocular pressure.

CONTRAINDICATIONS

Acetazolamide therapy is contraindicated in situations in which sodium and/or potassium blood serum levels are depressed, in cases of marked kidney and liver disease or dysfunction, in suprarenal gland failure, and in hyperchloremic acidosis. It is contraindicated in patients with cirrhosis because of the risk of development of hepatic encephalopathy.

Long-term administration of acetazolamide is contraindicated in patients with chronic noncongestive angle-closure glaucoma since it may permit organic closure of the angle to occur while the worsening glaucoma is masked by lowered intraocular pressure.

WARNINGS

Fatalities have occurred, although rarely, due to severe reactions to sulfonamides including Stevens-Johnson syndrome, toxic epidermal necrolysis, fulminant hepatic necrosis, agranulocytosis, aplastic anemia, and other blood dyscrasias. Sensitizations may recur when a sulfonamide is readministered irrespective of the route of administration. If signs of hypersensitivity or other serious reactions occur, discontinue use of this drug. Caution is advised for patients receiving concomitant high-dose aspirin an acetazolamide, as anorexia, tachypnea, lethargy, coma and death have been reported.

PRECAUTIONS

General

Increasing the dose does not increase the diuresis and may increase the incidence of drowsiness and/or paresthesia. Increasing the dose often results in a decrease in diuresis. Under certain circumstances, however, very large doses have been given in conjunction with other diuretics in order to secure diuresis in complete refractory failure.

Information for Patients

Adverse reactions common to all sulfonamide derivatives may occur: anaphylaxis, fever, rash (including erythema multiforme, Stevens-Johnson syndrome, toxic epidermal necrolysis), crystalluria, renal calculus, bone marrow depression, thrombocytopenic purpura, hemolytic anemia, leukopenia, pancytopenia and agranulocytosis. Precaution is advised for early detection of such reactions and the drug should be discontinued and appropriate therapy instituted.

In patients with pulmonary obstruction or emphysema where alveolar ventilation may be impaired, acetazolamide which may precipitate or aggravate acidosis, should be used with caution.

Caution is advised for patients receiving concomitant high-dose aspirin and acetazolamide, as anorexia, tachypnea, lethargy, coma and death have been reported (see **WARNINGS**).

Laboratory Tests

To monitor for hematologic reactions common to all sulfonamides, it is recommended that a baseline CBC and platelet count be obtained on patients prior to initiating acetazolamide therapy and at regular intervals during therapy. If significant changes occur, early discontinuance and institution of appropriate therapy are important. Periodic monitoring of serum electrolytes is recommended.

Carcinogenesis, Mutagenesis, Impairment of Fertility

Long-term studies in animals to evaluate the carcinogenic potential of acetazolamide have not been conducted. In a bacterial mutagenicity assay, acetazolamide was not mutagenic when evaluated with and without metabolic activation. The drug had no effect on fertility when administered in the diet to male and female rats at a daily intake of up to 4 times the recommended human dose of 1000 mg in a 50 kg individual.

Pregnancy: Teratogenic Effects: Pregnancy Category C

Acetazolamide, administered orally or parenterally, has been shown to be teratogenic (defects of the limbs) in mice, rats, hamsters and rabbits. There are no adequate and well-controlled studies in pregnant women. Acetazolamide should be used in pregnancy only if the potential benefit justifies the potential risk to the fetus.

Nursing Mothers

Because of the potential for serious adverse reaction in nursing infants from acetazolamide, a decision should be made whether to discontinue nursing or to discontinue the drug taking into account the

importance of the drug to the mother.

Description of Oral ACZ Drug Product

Manufactured by:
Emcure Pharmaceuticals USA, Inc.
21-B Cotters Lane
East Brunswick, NJ 08816

Distributed by:
Zydus Pharmaceuticals USA Inc.
Princeton, NJ 08534

Repackaged by:
American Health Packaging
Columbus, Ohio 43217

Package Insert
ACETAZOLAMIDE- acetazolamide capsule, extended release
American Health Packaging

Acetazolamide Extended-Release Capsules

Rx Only

DESCRIPTION

Acetazolamide extended-release capsules are an inhibitor of the enzyme carbonic anhydrase.

Acetazolamide is a white to faintly yellowish white crystalline, odorless powder, weakly acidic, very slightly soluble in water and slightly soluble in alcohol. The chemical name for acetazolamide is N-(5-Sulfamoyl-1,3,4-thiadiazol-2-yl)acetamide and has the following chemical structure:

MW 222.24 C4H6N4O3S2

Each acetazolamide extended-release capsule intended for oral administration contains 500 mg of acetazolamide. In addition, each capsule contains the following inactive ingredients: ammonio methacrylate copolymer dispersion type A and B, FD&C yellow #6, gelatin, microcrystalline cellulose, sodium lauryl sulfate, talc and titanium dioxide. The capsule is printed with black pharmaceutical ink which contains black iron oxide as a coloring agent.

CLINICAL PHARMACOLOGY

Acetazolamide is a potent carbonic anhydrase inhibitor, effective in the control of fluid secretion (e.g., some types of glaucoma), in the treatment of certain convulsive disorders (e.g., epilepsy) and in the promotion of diuresis in instances of abnormal fluid retention (e.g., cardiac edema).

Acetazolamide is not a mercurial diuretic. Rather, it is a non-bacteriostatic sulfonamide possessing a chemical structure and pharmacological activity distinctly different from the bacteriostatic

sulfonamides.

Acetazolamide is an enzyme inhibitor that acts specifically on carbonic anhydrase, the enzyme that catalyzes the reversible reaction involving the hydration of carbon dioxide and the dehydration of carbonic acid. In the eye, this inhibitory action of acetazolamide decreases the secretion of aqueous humor and results in a drop in intraocular pressure, a reaction considered desirable in cases of glaucoma and even in certain non-glaucomatous conditions. Evidence seems to indicate that acetazolamide has utility as an adjuvant in treatment of certain dysfunctions of the central nervous system (e.g., epilepsy). Inhibition of carbonic anhydrase in this area appears to retard abnormal, paroxysmal, excessive discharge from central nervous system neurons. The diuretic effect of acetazolamide is due to its action in the kidney on the reversible reaction involving hydration of carbon dioxide and dehydration of carbonic acid. The result is renal loss of HCO3 ion, which carries out sodium, water, and potassium. Alkalinization of the urine and promotion of diuresis are thus affected. Alteration in ammonia metabolism occurs due to increased reabsorption of ammonia by the renal tubules as a result of urinary alkalinization.

Acetazolamide extended-release capsules provide prolonged action to inhibit aqueous humor secretion for 18 to 24 hours after each dose, whereas tablets act for only eight to 12 hours. The prolonged continuous effect of acetazolamide permits a reduction in dosage frequency.

Plasma concentrations of acetazolamide peak from three to six hours after administration of acetazolamide extended-release capsules, compared to one to four hours with tablets. Food does not affect bioavailability of acetazolamide extended-release capsules.

Placebo-controlled clinical trials have shown that prophylactic administration of acetazolamide at a dose of 250 mg every eight to 12 hours (or a 500 mg controlled-release capsule once daily) before and during rapid ascent to altitude results in fewer and/or less severe symptoms of acute mountain sickness (AMS) such as headache, nausea, shortness of breath, dizziness, drowsiness, and fatigue. Pulmonary function (e.g., minute ventilation, expired vital capacity, and peak flow) is greater in the acetazolamide treated group, both in subjects with AMS and asymptomatic subjects. The acetazolamide treated climbers also had less difficulty in sleeping.

INDICATIONS AND USAGE

For adjunctive treatment of: chronic simple (open-angle) glaucoma, secondary glaucoma, and preoperatively in acute angle-closure glaucoma where delay of surgery is desired in order to lower intraocular pressure. Acetazolamide extended-release capsules are also indicated for the prevention or amelioration of symptoms associated with acute mountain sickness despite gradual ascent.

CONTRAINDICATIONS

Hypersensitivity to acetazolamide or any excipients in the formulation. Since acetazolamide is a sulfonamide derivative, cross sensitivity between acetazolamide, sulfonamides and other sulfonamide derivatives is possible.

Acetazolamide therapy is contraindicated in situations in which sodium and/or potassium blood serum levels are depressed, in cases of marked kidney and liver disease or dysfunction, in suprarenal gland failure, and in hyperchloremic acidosis. It is contraindicated in patients with cirrhosis because of the risk of development of hepatic encephalopathy.

Long-term administration of acetazolamide is contraindicated in patients with chronic non-congestive angle-closure glaucoma since it may permit organic closure of the angle to occur while the worsening

glaucoma is masked by lowered intraocular pressure.

WARNINGS

Fatalities have occurred, although rarely, due to severe reactions to sulfonamides including Stevens-Johnson syndrome, toxic epidermal necrolysis, fulminant hepatic necrosis, anaphylaxis, agranulocytosis, aplastic anemia, and other blood dyscrasias. Sensitizations may recur when a sulfonamide is readministered irrespective of the route of administration. If signs of hypersensitivity or other serious reactions occur, discontinue use of this drug.

Caution is advised for patients receiving concomitant high-dose aspirin and acetazolamide, as anorexia, tachypnea, lethargy, metabolic acidosis, coma, and death have been reported.

PRECAUTIONS

General

Increasing the dose does not increase the diuresis and may increase the incidence of drowsiness and/or paresthesia. Increasing the dose often results in a decrease in diuresis. Under certain circumstances, however, very large doses have been given in conjunction with other diuretics in order to secure diuresis in complete refractory failure.

Information for Patients

Adverse reactions common to all sulfonamide derivatives may occur: anaphylaxis, fever, rash (including erythema multiforme, Steven-Johnson syndrome, toxic epidermal necrolysis), crystalluria, renal calculus, bone marrow depression, thrombocytopenic purpura, hemolytic anemia, leukopenia, pancytopenia, and agranulocytosis. Caution is advised for early detection of such reactions and the drug should be discontinued and appropriate therapy instituted.

In patients with pulmonary obstruction or emphysema where alveolar ventilation may be impaired, acetazolamide which may precipitate or aggravate acidosis should be used with caution. Gradual ascent is desirable to try to avoid acute mountain sickness. If rapid ascent is undertaken and acetazolamide is used, it should be noted that such use does not obviate the need for prompt descent if severe forms of high altitude sickness occur, i.e., high altitude pulmonary edema (HAPE) or high altitude cerebral edema.

Caution is advised for patients receiving concomitant high-dose aspirin and acetazolamide, as anorexia, tachypnea, lethargy, metabolic acidosis, coma, and death have been reported (see WARNINGS).

Both increases and decreases in blood glucose have been described in patients treated with acetazolamide. This should be taken into consideration in patients with impaired glucose tolerance or diabetes mellitus.

Acetazolamide treatment may cause electrolyte imbalances, including hyponatremia and hypokalemia, as well as metabolic acidosis. Therefore, periodic monitoring of serum electrolytes is recommended. Particular caution is recommended in patients with conditions that are associated with, or predispose a patient to, electrolyte and acid/base imbalances, such as patients with impaired renal function (including elderly patients; see PRECAUTIONS, PRECAUTIONS, Geriatric Use), patients with diabetes mellitus, and patients with impaired alveolar ventilation.

Some adverse reactions to acetazolamide, such as drowsiness, fatigue, and myopia, may impair the ability to drive and operate machinery.

Laboratory Tests

To monitor for hematologic reactions common to all sulfonamides, it is recommended that a baseline CBC and platelet count be obtained on patients prior to initiating acetazolamide therapy and at regular intervals during therapy. If significant changes occur, early discontinuance and institution of appropriate therapy are important. Periodic monitoring of serum electrolytes is recommended.

Drug Interactions

Aspirin:

See WARNINGS
Acetazolamide modifies phenytoin metabolism with increased serum levels of phenytoin. This may increase or enhance the occurrence of osteomalacia in some patients receiving chronic phenytoin therapy. Caution is advised in patients receiving chronic concomitant therapy. By decreasing the gastrointestinal absorption of primidone, acetazolamide may decrease serum concentrations of primidone and its metabolites, with a consequent possible decrease in anticonvulsant effect. Caution is advised when beginning, discontinuing, or changing the dose of acetazolamide in patients receiving primidone.Because of possible additive effects with other carbonic anhydrase inhibitors, concomitant use is not advisable.
Acetazolamide may increase the effects of other folic acid antagonists.
Acetazolamide decreases urinary excretion of amphetamine and may enhance the magnitude and duration of their effect.
Acetazolamide reduces urinary excretion of quinidine and may enhance its effect.
Acetazolamide may prevent the urinary antiseptic effect of methenamine.
Acetazolamide increases lithium excretion and the lithium may be decreased.
Acetazolamide and sodium bicarbonate used concurrently increases the risk of renal calculus formation.
Acetazolamide may elevate cyclosporine levels.

Drug/Laboratory Test Interactions

Sulfonamides may give false negative or decreased values for urinary phenolsulfonphthalein and phenol red elimination values for urinary protein, serum non-protein, and serum uric acid.
Acetazolamide may produce an increased level of crystals in the urine.

Acetazolamide interferes with the HPLC method of assay for theophylline. Interference with the theophylline assay by acetazolamide depends on the solvent used in the extraction; acetazolamide may not interfere with other assay methods for theophylline.

Carcinogenesis, Mutagenesis, Impairment of Fertility

Long-term studies in animals to evaluate the carcinogenic potential of acetazolamide has not been conducted. In a bacterial mutagenicity assay, acetazolamide was not mutagenic when evaluated with and without metabolic activation.

The drug had no effect on fertility when administered in the diet to male and female rats at a daily intake of up to 4 times the recommended human dose of 1000 mg in a 50 kg individual.

Pregnancy
Teratogenic Effects
Pregnancy Category C:

Acetazolamide, administered orally or parenterally, has been shown to be teratogenic (defects of the limbs) in mice, rats, hamsters, and rabbits. There are no adequate and well-controlled studies in pregnant women. Acetazolamide should be used in pregnancy only if the potential benefit justifies the potential risk to the fetus.

Nursing Mothers

Because of the potential for serious adverse reactions in nursing infants from acetazolamide, a decision should be made whether to discontinue nursing or to discontinue the drug taking into account the importance of the drug to the mother. Acetazolamide should only be used by nursing women if the potential benefit justifies the potential risk to the child.

Pediatric Use

The safety and effectiveness of acetazolamide extended-release capsules in pediatric patients below the age of 12 years have not been established. Growth retardation has been reported in children receiving long-term therapy, believed secondary to chronic acidosis.

Geriatric Use

Metabolic acidosis, which can be severe, may occur in the elderly with reduced renal function.

In general, dose selection for an elderly patient should be cautious, usually starting at the low end of the dosing range, reflecting the greater frequency of decreased hepatic, renal, or cardiac function, and concomitant disease or other drug therapy.

ADVERSE REACTIONS
Body as a whole:
Headache, malaise, fatigue, fever, pain at injection site, flushing, growth retardation in children, flaccid paralysis, anaphylaxis.
Digestive:
Gastrointestinal disturbances such as nausea, vomiting, diarrhea.
Hematological/Lymphatic:
Blood dyscrasias such as aplastic anemia, agranulocytosis, leukopenia, thrombocytopenic purpura, melena.
Hepato-biliary disorders:
Abnormal liver function, cholestatic jaundice, hepatic insufficiency, fulminant hepatic necrosis
Metabolic/Nutritional:
Metabolic acidosis, electrolyte imbalance, including hypokalemia, hyponatremia, osteomalacia with long-term phenytoin therapy, loss of appetite, taste alteration, hyper/hypoglycemia
Nervous:
Drowsiness, paresthesia (including numbness and tingling of extremities and face), depression, excitement, ataxia, confusion, convulsions dizziness
Skin:
Allergic skin reactions including urticaria, photosensitivity, Stevens-Johnson syndrome, toxic epidermal necrolysis
Special senses:

Hearing disturbances, tinnitus, transient myopia
Urogenital:
Crystalluria, increased risk of nephrolithiasis with long-term therapy, hematuria, glycosuria, renal failure polyuria

OVERDOSAGE

No specific antidote is known. Treatment should be symptomatic and supportive.

Electrolyte imbalance, development of an acidotic state, and central nervous system effects might be expected to occur. Serum electrolyte levels (particularly potassium) and blood pH levels should be monitored.

Supportive measures are required to restore electrolyte and pH balance. The acidotic state can usually be corrected by the administration of bicarbonate.

Despite its high intraerythrocytic distribution and plasma protein binding properties, acetazolamide may be dialyzable. This may be particularly important in the management of acetazolamide overdosage when complicated by the presence of renal failure.

DOSAGE AND ADMINISTRATION

Glaucoma:

The recommended dosage is 1 capsule (500 mg) two times a day. Usually 1 capsule is administered in the morning and 1 capsule in the evening. It may be necessary to adjust the dose, but it has usually been found that dosage in excess of 2 capsules (1 g) does not produce an increased effect. The dosage should be adjusted with careful individual attention both to symptomatology and intraocular tension. In all cases, continuous supervision by a physician is advisable.

In those unusual instances where adequate control is not obtained by the twice-a-day administration of acetazolamide extended-release capsules, the desired control may be established by means of acetazolamide (tablets or parenteral). Use tablets or parenteral in accordance with the more frequent dosage schedules recommended for these dosage forms, such as 250 mg every four hours, or an initial dose of 500 mg followed by 250 mg or 125 mg every four hours, depending on the case in question.

Acute Mountain Sickness:

Dosage is 500 mg to 1000 mg daily, in divided doses using tablets or extended-release capsules as appropriate. In circumstances of rapid ascent, such as in rescue or military operations, the higher dose level of 1000 mg is recommended. It is preferable to initiate dosing 24 to 48 hours before ascent and to continue for 48 hours while at high altitude, or longer as necessary to control symptoms.

HOW SUPPLIED

Acetazolamide Extended-Release Capsules, 500 mg are white to off-white pellets filled in empty hard gelatin capsules with orange opaque cap imprinted with "EP" in black ink and white opaque body imprinted with "107" in black ink and are supplied as follows:

Unit dose packages of 30 (3x10) NDC 68084-401-21

Storage and Drug Accountability

Acetazolamide tablets will be stored at room temperature (between 68°F to 77°F) as suggested by the manufacturer. Intravenous formulation of ACZ will be stored as lyophilized powder at the temperature specified above in the Hermann Research pharmacy. Lyophilized powder will be reconstituted prior to infusion by the pharmacy. All study medication will be stored at the Hermann Research pharmacy and dispensed as needed during study.

TREATMENT

Rationale for Selection of Dose

The ACZ challenge test, using 1000 mg IV ACZ, is a commonly utilized clinical tool in evaluating patients with chronic intracerebral occlusive disease and is a reliable predictor of critically reduced cerebral perfusion (Vagal et al., 2009). Oral doses as high as 4000 mg daily have been previously used in the NORDIC clinical trial (NORDIC Idiopathic Intracranial Hypertension Study Group Writing Committee et al., 2014).

Participants in this study will be treated with the highest tolerated dose of oral ACZ extended release capsules shown to exert the greatest effect on cerebral perfusion. This dose will also be utilized for an anticipated subsequent large phase 3 trial to determine clinical efficacy.

Study Drug Formulations

FDA approved extended release ACZ tablets from American Health will be used for “Treatment A”. Commercially available placebo capsules manufactured by American Health composed entirely of lactose monohydrate filler that will be used for “Treatment B”.

Study Drug Administration

“Treatment A” will be obtained directly from American Health and stored at MHHS pharmacy at Memorial Hermann Hospital. “Treatment B” is commercially available directly from American Health and will be shipped to MHHS pharmacy, shipped to CPHS pharmacy and stored. For stage 3, patients will be given either “Treatment A” or “Treatment B”, based upon randomization, in sufficient quantities for each study visit. At the end of each study visit, patients will be asked to return any unused capsules. Unused capsules will be counted and stored in a secure facility at the McGovern Medical School, UTHealth.

Subjects with known hypersensitivity to sulfonamides or derivative drugs will be excluded from study participation (see section 3.4 for complete exclusion criteria). Subjects will be informed of common adverse reactions to sulfonamides including anaphylaxis, fever, rash (including erythema multiforme, Stevens-Johnson syndrome, toxic epidermal necrolysis), crystalluria, renal calculus, bone marrow depression, thrombocytopenic purpura, hemolytic anemia, leukopenia, pancytopenia and agranulocytosis. The drug will be discontinued and appropriate therapy instituted for any of these adverse reactions.

Specific Restrictions / Requirements

Subjects will be required to discontinue use of any frequently used over the counter medication, vitamin or other supplement one week prior to treatment with ACZ and for the duration of the study.

Blinding

Stage 1a to 2b are unblinded stages. Stage 3 is the only double-blinded portion of this study. Both subjects and raters will remain blinded to treatment randomization.Common AE such as paresthesias, particularly a “tingling” feeling in the extremities, and gastrointestinal disturbances such as nausea, vomiting and diarrhea typically occur early in treatment and might be more pronounced in subjects initially randomized to ACZ. Subjects will be encouraged to avoid disclosing any AE so as to maintain the blind for clinical rater(s). Imaging rater(s) will not have direct contact with study subjects and no potential for unblinding is present.

Patients with intolerable paraesthesia, change in taste, accelerated change in blood pressure or fluid retention will be withdrawn from the study. Patients with persistent AEs will be referred to their primary care physician for continued monitoring and treatment using standard or care practices.

Concomitant therapy

Subjects will be encouraged to refrain from any over the counter medication, vitamin or other supplement including concomitant use of high-dose aspirin for analgesia. Subjects will be warned that an increased potential for serious AE has been reported in patients taking both ACZ and high-dose aspirin.

Subjects will be required, as stated in inclusion criteria (see section 3.3,) to be stable on any FDA-approved DMT.

SAFETY MEASUREMENTS

Definitions

The descriptions and grading scales found in the NCI Common Terminology Criteria for Adverse Events v 3.0 (CTCAE; published June 10, 2013) will be utilized for AE reporting.

Attribution of the AE:
- Definite – The AE is clearly related to the study treatment
- Probable – The AE is likely related to the study treatment
- Possible – The AE may be related to the study treatment
- Unlikely – The AE is doubtfully related to the study treatment
- Unrelated – The AE is clearly NOT related to the study treatment

AEs should be recorded and graded 1 to 5 according to the CTCAE grades provided below:
Grade 1 = Mild adverse event
Grade 2 = Moderate adverse event
Grade 3 = Severe and undesirable adverse event
Grade 4 = Life-threatening or disabling adverse event
Grade 5 = Death

For additional information, consult the National Cancer Institute’s Common Terminology Criteria for Adverse Events v3.0 (published June 10, 2003) at the following URL:
http://ctep.cancer.gov/reporting/ctc.html

An AE is defined as any untoward medical occurrence in a patient administered a pharmaceutical

product. Adverse events are to be recorded in the clinical chart. Severity, action taken, and relationship will be recorded as noted in the chart.

Serious Adverse Event (SAE) is defined as any untoward medical occurrence resulting in hospitalization or additional medical intervention in a patient administered a pharmaceutical product. SAE is to be recorded in the clinical chart. Severity, action taken, and relationship will be recorded as noted in the chart.

A comprehensive list of AEs provides previously reported and/or potential AEs associated with ACZ. Common AEs are in **bold**. They include **paresthesias**, particularly a “tingling” feeling in the extremities, hearing dysfunction or tinnitus, loss of appetite, **taste alteration** and gastrointestinal disturbances such as **nausea, vomiting, dyspepsia and diarrhea**, polyuria, and occasional instances of **drowsiness/fatigue** and confusion. Metabolic acidosis and electrolyte imbalance may occur. Transient myopia has been reported though this condition invariably subsides upon diminution or discontinuance of the medication.
Other occasional adverse reactions include urticaria, melena, hematuria, glycosuria, hepatic insufficiency, flaccid paralysis, photosensitivity and convulsions.

Collecting, Recording and Reporting of Adverse Events

Recording AEs – The treating physician will be the principal investigator (John Lincoln). The treating physician will oversee subject management including all assessments and treatment of adverse events and disease relapses should they occur. The treating physician will make decisions regarding determining relatedness and grading severity of all AEs. The treating physician will be blinded to treatment arm unless necessary to assess safety concern.

Maintaining documentation and reporting AEs – The clinical coordinator (James Jemelka) will assist in subject management, maintain documentation including all subject case report forms (unanticipated problems tracking log) and will submit all reports to the IRB per UTSHC-Houston policy

Safety Monitoring Plan

This pilot study will determine the extent to which improved cerebral perfusion from oral ACZ therapy reduces the evolution of MS lesion into permanent black hole. Subjects will be monitored, as per protocol, by the treating physician for clinical MS activity. However, to our knowledge there are no case reports or other studies to suggest that therapy with ACZ will contribute to new lesion formation or clinical relapse. As such, we do not anticipate an increase in relapse frequency secondary to ACZ.

Subjects will be monitored by the treating physician for AEs and serious AEs (see section 9.1). The frequency and grade of AEs will be documented and treatment arm(s) adjusted or discontinued as needed. Subjects can withdraw consent for study at any point and for any reason during the trial.

ACZ is a well-established FDA approved therapy for various acute and chronic diseases. Patients generally tolerate drug without serious AEs. We have not observed any serious AE in our experience with IV or oral ACZ and do not anticipate any serious AE with oral ACZ used in this trial.

To ensure safety to participants, an independent 3-person DSMB made up of two individuals with expertise in vascular neurology and one with statistical or epidemiological background will be coordinated by the UTHealth CCTS Regulatory Group. The 3-member board will meet biannually to review enrolment, evaluate reasons for screen failure, detail protocol deviations and treatment duration for participants. In addition, they will review all adverse events.

DATA ANALYSIS

Data Quality Assurance

Each investigator in this study is experienced in the scientific method and have participated in or independently conducted clinical trials. All data during stage 2 of this study will be gathered by raters blinded to treatment.

Data Entry and Storage

As patients consent for the study they will be given a unique patient study code number that will be used to identify them in a database specifically constructed for the study. All necessary patient demographics will be collected for entry into the database by a study coordinator/data entry manager (James Jemelka). Information gathered on paper will be transferred to REDCap (Research Electronic Data CAPture), an electronic data capture software package available to academic institutions.

The MRI-Analysis Center (MRI-AC), directed by Dr. Wolinsky, has extensive experience with the management of large amounts of de-identified patient related data and images gathered for both local research studies and national and international studies on thousands of subjects, each with multiple sessions. All data is stored on the MRI-AC macpdbs1 server physically secured in the University Professional Tower Data Center located within virtual Zone 100 (contains servers with protected health information as well as other sensitive or critical data; no access may be initiated directly from the Internet to Zone 100; all servers in Zone 100 are geographically located in secure areas). All workstations physically located in the MRI-AC are confined to Zone 40 (contains the bulk of the computing resources geographically distributed throughout the UTHSCH campus; no access may be initiated directly from the Internet into Zone 40; protected health information must be encrypted; rules for traffic initiated form Zone 40 to other areas: Internet is allowed, Zone 20 is allowed, Zone 100 is restricted to defined servers and protocols). Only individuals who can authenticate to the VPN appliance connected to Zone 40 and have appropriate authorization privileges may access MRI-AC resources from the Internet. Port configurations of the firewalls separating the Zones are managed by the UTHSCH Information Security Staff. Port configuration is managed by a firewall database in accordance with firewall policies and procedures. The MRI-AC Director is the information owner of the macpdbs1 server and the workstations. The steward of the macpdbs1 server is the Data Center Operations and Services (DCOS) division of the UTHSCH Information Technology Department. The steward of the MRI-AC workstations is the Medical School Information Technology (MSIT) Office. We will utilize these resources to manage and store all data and digital images generated as part of this project.

SAMPLE SIZE AND STATISTICAL METHODS

Studies have shown between 10% - 15% decrease in cerebral perfusion in patients with relapsing MS *(Adhya et al., 2006)*. Therefore, increases in CBF of at least 15% might result in change to lesion evolution.

The primary aim of the proposed study is to evaluate the change in global CBF with ACZ, so the primary outcome is the change in global CBF after 24 weeks relative to pre-treatment baseline. Based on the preliminary data, we expect to see a 15% difference between the ACZ treatment and placebo groups on the improvement in global cerebral perfusion with standard deviation (SD) of 30%. We ran Monte Carlo simulations under a linear mixed effect model (same model as planned for analysis) and calculated a needed total sample size of n=50 to detect a group difference of 0.5 SD at the 24 weeks (phase 1) assuming a within-subject correlation of 0.5, a significance level of 0.05, and 90% power. We hypothesize a similar group difference in phase 2 (ACZ-ACZ group would not change between weeks 24 and 48 but delayed-start group would improve) and similar power to detect this effect. With 50 patients, for Aim 1.2, we will have 80% power to detect an average correlation as low as 0.39 between CBF and MD or FA. Considering a dropout rate of 18%, we need to recruit 60 participants. Aims 2 are mainly exploratory and parameters derived from this study will be used to help design future larger scale confirmatory studies.

All analyses will be intent-to-treat. To summarize the data, descriptive statistics will be used. Demographics and baseline characteristics at randomization will be summarized for each treatment arm. Generalized linear mixed models will be used to analyze all outcomes and will include treatment group and time, group-by-time interaction, and disease duration (stratifying variable) as covariates, and a random intercept to account for within-patient correlation.

For the primary outcome, we will focus on comparing the treatment groups in phase 1. A linear mixed model will be used to compare the estimated change in global CBF between baseline and week 24 in the ACZ and delayed-start groups. A secondary analysis will study ACZ treatment effect on CBF in different regions by treating regions as clusters in the linear mixed model. If normality of CBF outcome does not hold, an appropriate transformation or different outcome distribution will be used.

For the secondary endpoint at 48 weeks (phase 2), a similar mixed model will be used to compare changes in global CBF between weeks 24 and 48 weeks in the early-start and delayed-start groups. The model will include the same terms as for phase 1.

Secondary outcomes considers repeated measures of MD or FA at different time in different regions. Linear mixed models will be used to evaluate changes in MD and FA from baseline (24 weeks for delayed-start) to 24 weeks (48 weeks for delayed-start) after ACZ therapy. These changes will be compared between regions with and without increased CBF (>15% difference from baseline). Terms in the model will be as for CBF with the addition of increased CBF (yes/no) for the region. Mixed models will also be used to compare MD and FA changes between white matter areas with and without lesions.

Logistic or linear mixed models will be used to evaluate change from baseline to after 24 week ACZ therapy (using phase 1 and phase 2 data) in the tertiary composite change outcome. Models will include the same terms as for analyses of the primary outcome. We will evaluate associations between changes in clinical outcomes and changes in CBF, CBV, and MTT by including each of these variables (one at a time) as a time-varying covariate.

As this trial uses a small sample size, we would not expect statistically significant treatment effects on clinical disability though this exploratory measure should be useful in power calculation for the anticipated larger, randomized clinical trial.

ETHICAL CONSIDERATIONS

Informed Consent

The informed consent form is a method of providing information regarding the trial to a prospective participant and allows for an informed decision about participation in the study. All participants (or their legally acceptable representative) must read, sign, and date a consent form before participating in the study, taking study drug, and/or undergoing any study-specific procedures. If a participant does not speak and read English, the consent materials will be translated into the Spanish.

The informed consent form will be updated or revised whenever important new safety information is available, whenever the protocol is amended, and/or whenever any new information becomes available that may affect a subject's participation in the trial.

A copy of the informed consent form will be given to a prospective participant for review. The principle investigator (J.L.), in the presence of a witness, will review the consent form, discuss potential AEs associated with ACZ therapy and, when necessary, discuss the imaging protocol to be utilized and answer questions. The participant will be informed that their participation is voluntary and that they may withdraw from the study at any time, for any reason.

IRB review

This protocol and the associated informed consent documents must be submitted to the IRB for review and approval.

Confidentiality of Data and Patient Records

As patients consent for the study they will be given a unique patient study code number that will be used to identify them in a database specifically constructed for the study. All necessary patient demographics will be collected for entry into the database by a study coordinator/data entry manager (James Jemelka).
Results of blood and urine tests obtained during the study period will also be coded to the patient and collected for entry into the database by a study coordinator/data entry manager. The study PI will review test results solely to determine subject exclusion or removal from the study and to document laboratory-specific AE.

PUBLICATIONS

The data or results of the completed study may become the subject of, or contained within, a refereed, reputable peer review journal.

RETENTION OF TRIAL DOCUMENTS

Records for all participants, including CRFs, all source documentation (containing evidence to study eligibility, history and physical findings, laboratory data, results of consultations, etc.) as well as IRB records and other regulatory documentation will be collected by the study coordinator/data entry manager (James Jemelka) and PI (John Lincoln) and will be stored on-site at UT Health. The PI will be the sole custodian of this information.

CLINICAL PROTOCOL – REFERENCES

Acetazolamide Tablets | Drug Summary | PDR.net [WWW Document], n.d. URL http://www.pdr.net/drug-summary/acetazolamide-tablets?druglabelid=667&id=637 (accessed 2.12.14).

Adhya, S., Johnson, G., Herbert, J., Jaggi, H., Babb, J.S., Grossman, R.I., Inglese, M., 2006. Pattern of hemodynamic impairment in multiple sclerosis: Dynamic susceptibility contrast perfusion MR imaging at 3.0 T. NeuroImage 33, 1029–1035. doi:10.1016/j.neuroimage.2006.08.008

Aslan, S., Xu, F., Wang, P.L., Uh, J., Yezhuvath, U.S., van Osch, M., Lu, H., 2010. Estimation of labeling efficiency in pseudocontinuous arterial spin labeling. Magn. Reson. Med. Off. J. Soc. Magn. Reson. Med. Soc. Magn. Reson. Med. 63, 765–771. doi:10.1002/mrm.22245

Barnett, M.H., Henderson, A.P.D., Prineas, J.W., 2006. The macrophage in MS: just a scavenger after all? Pathology and pathogenesis of the acute MS lesion. Mult. Scler. Houndmills Basingstoke Engl. 12, 121–132.

Barnett, M.H., Prineas, J.W., 2004. Relapsing and remitting multiple sclerosis: pathology of the newly forming lesion. Ann. Neurol. 55, 458–468. doi:10.1002/ana.20016

Caramanos, Z., Francis, S.J., Narayanan, S., Lapierre, Y., Arnold, D.L., 2012. Large, nonplateauing relationship between clinical disability and cerebral white matter lesion load in patients with multiple sclerosis. Arch. Neurol. 69, 89–95. doi:10.1001/archneurol.2011.765

Cotton, F., Weiner, H.L., Jolesz, F.A., Guttmann, C.R.G., 2003. MRI contrast uptake in new lesions in relapsing-remitting MS followed at weekly intervals. Neurology 60, 640–646.

Dai, W., Garcia, D., de Bazelaire, C., Alsop, D.C., 2008. Continuous flow-driven inversion for arterial spin labeling using pulsed radio frequency and gradient fields. Magn. Reson. Med. Off. J. Soc. Magn. Reson. Med. Soc. Magn. Reson. Med. 60, 1488–1497. doi:10.1002/mrm.21790

Debernard, L., Melzer, T.R., Van Stockum, S., Graham, C., Wheeler-Kingshott, C.A., Dalrymple-Alford, J.C., Miller, D.H., Mason, D.F., 2013. Reduced grey matter perfusion without volume loss in early relapsing-remitting multiple sclerosis. J. Neurol. Neurosurg. Psychiatry. doi:10.1136/jnnp-2013-305612

Filippi, M., 2000. Enhanced magnetic resonance imaging in multiple sclerosis. Mult. Scler. Houndmills Basingstoke Engl. 6, 320–326.

Friberg, L., Kastrup, J., Rizzi, D., Jensen, J.B., Lassen, N.A., 1990. Cerebral blood flow and end-tidal PCO2 during prolonged acetazolamide treatment in humans. Am. J. Physiol. 258, H954-959.

Ghassemi, R., Narayanan, S., Banwell, B., Sled, J.G., Shroff, M., Arnold, D.L., Canadian Pediatric Demyelinating Disease Network, 2014. Quantitative determination of regional lesion volume and distribution in children and adults with relapsing-remitting multiple sclerosis. PloS One 9, e85741. doi:10.1371/journal.pone.0085741

Giorgio, A., Stromillo, M.L., Bartolozzi, M.L., Rossi, F., Battaglini, M., De Leucio, A., Guidi, L., Maritato, P., Portaccio, E., Sormani, M.P., Amato, M.P., De Stefano, N., 2013. Relevance of hypointense brain MRI lesions for long-term worsening of clinical disability in relapsing multiple sclerosis. Mult. Scler. J. 20, 214–219. doi:10.1177/1352458513494490

Hartkamp, N.S., Hendrikse, J., van der Worp, H.B., de Borst, G.J., Bokkers, R.P.H., 2012. Time course of vascular reactivity using repeated phase-contrast MR angiography in patients with carotid artery stenosis. Stroke J. Cereb. Circ. 43, 553–556. doi:10.1161/STROKEAHA.111.637314

Helenius, J., Perkiö, J., Soinne, L., Østergaard, L., Carano, R.A.D., Salonen, O., Savolainen, S., Kaste, M., Aronen, H.J., Tatlisumak, T., 2003. Cerebral hemodynamics in a healthy population measured by dynamic susceptibility contrast MR imaging. Acta Radiol. Stockh. Swed. 1987 44, 538–546.

Holland, C.M., Charil, A., Csapo, I., Liptak, Z., Ichise, M., Khoury, S.J., Bakshi, R., Weiner, H.L., Guttmann, C.R.G., 2012. The relationship between normal cerebral perfusion patterns and

white matter lesion distribution in 1,249 patients with multiple sclerosis. J. Neuroimaging Off. J. Am. Soc. Neuroimaging 22, 129–136. doi:10.1111/j.1552-6569.2011.00585.x
Juurlink, B.H.J., 2013. The evidence for hypoperfusion as a factor in multiple sclerosis lesion development. Mult. Scler. Int. 2013, 598093. doi:10.1155/2013/598093
Koch-Henriksen, N., Sørensen, P.S., 2010. The changing demographic pattern of multiple sclerosis epidemiology. Lancet Neurol. 9, 520–532. doi:10.1016/S1474-4422(10)70064-8
Kurtzke, J.F., 1983. Rating neurologic impairment in multiple sclerosis: an expanded disability status scale (EDSS). Neurology 33, 1444–1452.
Lassmann, H., 2011. Review: The architecture of inflammatory demyelinating lesions: implications for studies on pathogenesis: Active lesions in multiple sclerosis. Neuropathol. Appl. Neurobiol. 37, 698–710. doi:10.1111/j.1365-2990.2011.01189.x
Leary, S.M., 2005. Multiple sclerosis: diagnosis and the management of acute relapses. Postgrad. Med. J. 81, 302–308. doi:10.1136/pgmj.2004.029413
Lim, L.-L., Foldvary, N., Mascha, E., Lee, J., 2001. Acetazolamide in Women with Catamenial Epilepsy. Epilepsia 42, 746–749. doi:10.1046/j.1528-1157.2001.33600.x
Meier, D.S., Guttmann, C.R.G., 2006. MRI time series modeling of MS lesion development. NeuroImage 32, 531–537. doi:10.1016/j.neuroimage.2006.04.181
Narayana, P.A., Doyle, T.J., Lai, D., Wolinsky, J.S., 1998. Serial proton magnetic resonance spectroscopic imaging, contrast-enhanced magnetic resonance imaging, and quantitative lesion volumetry in multiple sclerosis. Ann. Neurol. 43, 56–71. doi:10.1002/ana.410430112
Narayana, P.A., Zhou, Y., Hasan, K.M., Datta, S., Sun, X., Wolinsky, J.S., 2014. Hypoperfusion and T1-hypointense lesions in white matter in multiple sclerosis. Mult. Scler. Houndmills Basingstoke Engl. 20, 365–373. doi:10.1177/1352458513495936
NORDIC Idiopathic Intracranial Hypertension Study Group Writing Committee, Wall, M., McDermott, M.P., Kieburtz, K.D., Corbett, J.J., Feldon, S.E., Friedman, D.I., Katz, D.M., Keltner, J.L., Schron, E.B., Kupersmith, M.J., 2014. Effect of acetazolamide on visual function in patients with idiopathic intracranial hypertension and mild visual loss: the idiopathic intracranial hypertension treatment trial. JAMA J. Am. Med. Assoc. 311, 1641–1651. doi:10.1001/jama.2014.3312
Orton, S.-M., Herrera, B.M., Yee, I.M., Valdar, W., Ramagopalan, S.V., Sadovnick, A.D., Ebers, G.C., Canadian Collaborative Study Group, 2006. Sex ratio of multiple sclerosis in Canada: a longitudinal study. Lancet Neurol. 5, 932–936. doi:10.1016/S1474-4422(06)70581-6
Ota, M., Sato, N., Nakata, Y., Ito, K., Kamiya, K., Maikusa, N., Ogawa, M., Okamoto, T., Obu, S., Noda, T., Araki, M., Yamamura, T., Kunugi, H., 2013. Abnormalities of cerebral blood flow in multiple sclerosis: a pseudocontinuous arterial spin labeling MRI study. Magn. Reson. Imaging 31, 990–995. doi:10.1016/j.mri.2013.03.016
Papadaki, E.Z., Mastorodemos, V.C., Amanakis, E.Z., Tsekouras, K.C., Papadakis, A.E., Tsavalas, N.D., Simos, P.G., Karantanas, A.H., Plaitakis, A., Maris, T.G., 2012. White matter and deep gray matter hemodynamic changes in multiple sclerosis patients with clinically isolated syndrome. Magn. Reson. Med. Off. J. Soc. Magn. Reson. Med. Soc. Magn. Reson. Med. 68, 1932–1942. doi:10.1002/mrm.24194
Papadaki, E.Z., Simos, P.G., Panou, T., Mastorodemos, V.C., Maris, T.G., Karantanas, A.H., Plaitakis, A., 2013. Hemodynamic evidence linking cognitive deficits in clinically isolated syndrome to regional brain inflammation. Eur. J. Neurol. Off. J. Eur. Fed. Neurol. Soc. doi:10.1111/ene.12338
Papadopoulos, K., Tozer, D.J., Fisniku, L., Altmann, D.R., Davies, G., Rashid, W., Thompson, A.J., Miller, D.H., Chard, D.T., 2010. TI-relaxation time changes over five years in relapsing-remitting multiple sclerosis. Mult. Scler. Houndmills Basingstoke Engl. 16, 427–433. doi:10.1177/1352458509359924

Parry, A., Clare, S., Jenkinson, M., Smith, S., Palace, J., Matthews, P.M., 2002. White matter and lesion T1 relaxation times increase in parallel and correlate with disability in multiple sclerosis. J. Neurol. 249, 1279–1286. doi:10.1007/s00415-002-0837-7

Polman, C.H., Reingold, S.C., Banwell, B., Clanet, M., Cohen, J.A., Filippi, M., Fujihara, K., Havrdova, E., Hutchinson, M., Kappos, L., Lublin, F.D., Montalban, X., O'Connor, P., Sandberg-Wollheim, M., Thompson, A.J., Waubant, E., Weinshenker, B., Wolinsky, J.S., 2011. Diagnostic criteria for multiple sclerosis: 2010 Revisions to the McDonald criteria. Ann. Neurol. 69, 292–302. doi:10.1002/ana.22366

Reingold, S.C., 2002. Prevalence estimates for MS in the United States and evidence of an increasing trend for women. Neurology 59, 294; author reply 294-295.

Renoux, C., Vukusic, S., Mikaeloff, Y., Edan, G., Clanet, M., Dubois, B., Debouverie, M., Brochet, B., Lebrun-Frenay, C., Pelletier, J., Moreau, T., Lubetzki, C., Vermersch, P., Roullet, E., Magy, L., Tardieu, M., Suissa, S., Confavreux, C., Adult Neurology Departments KIDMUS Study Group, 2007. Natural history of multiple sclerosis with childhood onset. N. Engl. J. Med. 356, 2603–2613. doi:10.1056/NEJMoa067597

Sahraian, M.A., Radue, E.-W., Haller, S., Kappos, L., 2010. Black holes in multiple sclerosis: definition, evolution, and clinical correlations. Acta Neurol. Scand. 122, 1–8. doi:10.1111/j.1600-0404.2009.01221.x

Stewart, W.C., Halper, L.K., Johnson-Pratt, L., Polis, A., Hartenbaum, D., 2002. Tolerability and efficacy of dorzolamide versus acetazolamide added to timolol. J. Ocul. Pharmacol. Ther. Off. J. Assoc. Ocul. Pharmacol. Ther. 18, 211–220. doi:10.1089/108076802760116133

Sullivan, H.G., Kingsbury, T.B., 4th, Morgan, M.E., Jeffcoat, R.D., Allison, J.D., Goode, J.J., McDonnell, D.E., 1987. The rCBF response to Diamox in normal subjects and cerebrovascular disease patients. J. Neurosurg. 67, 525–534. doi:10.3171/jns.1987.67.4.0525

Thaler, C., Faizy, T.D., Sedlacik, J., Holst, B., Stürner, K., Heesen, C., Stellmann, J.-P., Fiehler, J., Siemonsen, S., 2017. T1 Recovery Is Predominantly Found in Black Holes and Is Associated with Clinical Improvement in Patients with Multiple Sclerosis. AJNR Am. J. Neuroradiol. 38, 264–269. doi:10.3174/ajnr.A5004

Trapp, B.D., Stys, P.K., 2009. Virtual hypoxia and chronic necrosis of demyelinated axons in multiple sclerosis. Lancet Neurol. 8, 280–291. doi:10.1016/S1474-4422(09)70043-2

Truyen, L., van Waesberghe, J.H., van Walderveen, M.A., van Oosten, B.W., Polman, C.H., Hommes, O.R., Adèr, H.J., Barkhof, F., 1996. Accumulation of hypointense lesions ("black holes") on T1 spin-echo MRI correlates with disease progression in multiple sclerosis. Neurology 47, 1469–1476.

Tullman, M.J., 2013. A review of current and emerging therapeutic strategies in multiple sclerosis. Am. J. Manag. Care 19, S21-27.

Vagal, A.S., Leach, J.L., Fernandez-Ulloa, M., Zuccarello, M., 2009. The Acetazolamide Challenge: Techniques and Applications in the Evaluation of Chronic Cerebral Ischemia. Am. J. Neuroradiol. 30, 876–884. doi:10.3174/ajnr.A1538

van Walderveen, M.A., Barkhof, F., Pouwels, P.J., van Schijndel, R.A., Polman, C.H., Castelijns, J.A., 1999a. Neuronal damage in T1-hypointense multiple sclerosis lesions demonstrated in vivo using proton magnetic resonance spectroscopy. Ann. Neurol. 46, 79–87.

van Walderveen, M.A., Kamphorst, W., Scheltens, P., van Waesberghe, J.H., Ravid, R., Valk, J., Polman, C.H., Barkhof, F., 1998. Histopathologic correlate of hypointense lesions on T1-weighted spin-echo MRI in multiple sclerosis. Neurology 50, 1282–1288.

van Walderveen, M.A., Truyen, L., van Oosten, B.W., Castelijns, J.A., Lycklama à Nijeholt, G.J., van Waesberghe, J.H., Polman, C., Barkhof, F., 1999b. Development of hypointense lesions on T1-weighted spin-echo magnetic resonance images in multiple sclerosis: relation to inflammatory activity. Arch. Neurol. 56, 345–351.

Walsh, A.J., Blevins, G., Lebel, R.M., Seres, P., Emery, D.J., Wilman, A.H., 2014. Longitudinal MR imaging of iron in multiple sclerosis: an imaging marker of disease. Radiology 270, 186–196. doi:10.1148/radiol.13130474

Warntjes, J.B.M., Engström, M., Tisell, A., Lundberg, P., 2013. Brain characterization using normalized quantitative magnetic resonance imaging. PloS One 8, e70864. doi:10.1371/journal.pone.0070864

Wong, E.C., 2007. Vessel-encoded arterial spin-labeling using pseudocontinuous tagging. Magn. Reson. Med. Off. J. Soc. Magn. Reson. Med. Soc. Magn. Reson. Med. 58, 1086–1091. doi:10.1002/mrm.21293

Wu, W.-C., Fernández-Seara, M., Detre, J.A., Wehrli, F.W., Wang, J., 2007. A theoretical and experimental investigation of the tagging efficiency of pseudocontinuous arterial spin labeling. Magn. Reson. Med. Off. J. Soc. Magn. Reson. Med. Soc. Magn. Reson. Med. 58, 1020–1027. doi:10.1002/mrm.21403

Wuerfel, J., Bellmann-Strobl, J., Brunecker, P., Aktas, O., McFarland, H., Villringer, A., Zipp, F., 2004. Changes in cerebral perfusion precede plaque formation in multiple sclerosis: a longitudinal perfusion MRI study. Brain J. Neurol. 127, 111–119. doi:10.1093/brain/awh007