2018

DUAL LOX/COX INHIBITION: A NOVEL STRATEGY TO PREVENT NEUROVASCULAR LEAKAGE IN EPILEPSY

Brent S. Sokola
University of Kentucky, brent.sokola@uky.edu
Author ORCID Identifier:
https://orcid.org/0000-0001-9452-6190
Digital Object Identifier: https://doi.org/10.13023/ETD.2018.159

STUDENT AGREEMENT:

I represent that my thesis or dissertation and abstract are my original work. Proper attribution has been given to all outside sources. I understand that I am solely responsible for obtaining any needed copyright permissions. I have obtained needed written permission statement(s) from the owner(s) of each third-party copyrighted matter to be included in my work, allowing electronic distribution (if such use is not permitted by the fair use doctrine) which will be submitted to UKnowledge as Additional File.

I hereby grant to The University of Kentucky and its agents the irrevocable, non-exclusive, and royalty-free license to archive and make accessible my work in whole or in part in all forms of media, now or hereafter known. I agree that the document mentioned above may be made available immediately for worldwide access unless an embargo applies.

I retain all other ownership rights to the copyright of my work. I also retain the right to use in future works (such as articles or books) all or part of my work. I understand that I am free to register the copyright to my work.

REVIEW, APPROVAL AND ACCEPTANCE

The document mentioned above has been reviewed and accepted by the student's advisor, on behalf of the advisory committee, and by the Director of Graduate Studies (DGS), on behalf of the program; we verify that this is the final, approved version of the student's thesis including all changes required by the advisory committee. The undersigned agree to abide by the statements above.

Brent S. Sokola, Student

Dr. Björn Bauer, Major Professor

Dr. David Feola, Director of Graduate Studies

DUAL LOX/COX INHIBITION: A NOVEL STRATEGY TO PREVENT NEUROVASCULAR LEAKAGE IN EPILEPSY

THESIS

A thesis submitted in partial fulfillment of the
requirements for the degree of Master of Science
in the College of Pharmacy at the University of Kentucky

By

Brent Scot Sokola

Lexington, Kentucky

Director: Dr. Björn Bauer, Associate Professor of Pharmaceutical Sciences

Lexington, Kentucky

2018

ABSTRACT OF THESIS

DUAL LOX/COX INHIBITION: A NOVEL STRATEGY TO PREVENT NEUROVASCULAR LEAKAGE IN EPILEPSY

Epilepsy affects 3.4 million patients in the USA and is characterized by recurring seizures. The blood-brain barrier is leaky in epilepsy and may contribute to seizure progression but the mechanisms which cause this leakage are not fully understood. We hypothesized that seizures trigger LOX- and COX-mediated blood-brain barrier leakage and that dual LOX/COX inhibition prevents barrier leakage *in vivo*. To test this hypothesis, we administered either the dual LOX/COX inhibitor licofelone or a combination of the 5-LOX inhibitor zileuton and the COX-2 inhibitor celecoxib to rats that experienced status epilepticus (SE). Serum and brain capillaries were isolated 48 hours after SE and serum S100β levels were measured and Texas Red™ leakage rates were determined. Dual inhibition of 5-LOX and COX prevented serum S100β elevations observed in SE rats in a dose-dependent manner with licofelone. Inhibition of 5-LOX and COX-2 with zileuton and celecoxib completely prevented serum S100β elevation. Texas Red™ leakage rates for SE rats were also reduced in a dose-depended manner with licofelone and reduced to control rates with zileuton and celecoxib. These data support our hypothesis that seizure-induced blood-brain barrier leakage is mediated by LOX and COX, and inhibition of these enzymes prevents barrier leakage.

Keywords or Phrases: blood-brain barrier, epilepsy, blood-brain barrier leakage, barrier dysfunction, 5-LOX, COX-2

Brent Scot Sokola

19 March 2018

DUAL LOX/COX INHIBITION: A NOVEL STRATEGY TO PREVENT NEUROVASCULAR LEAKAGE IN EPILEPSY

By

Brent Scot Sokola

Björn Bauer, PhD
Director of Thesis

David Feola, PharmD, PhD, BCPS
Director of Graduate Studies

19 March 2018

ACKNOWLEDGMENTS

I would first like to thank my thesis advisor Dr. Björn Bauer of the University of Kentucky College of Pharmacy. Dr. Bauer has been a mentor to me for many years inside and outside of the laboratory. His continued support has made me a better scientist and is an outstanding professional example to follow.

I would also like to thank Dr. Anika Hartz whose close collaboration was integral to the production of the data enclosed in this thesis and who has also supported my growth over many years.

To my lab mates, I would like to thank you for your assistance with my experiments and for always maintaining a collegial spirit in the lab.

I would also like to acknowledge my committee members Dr. James Pauly of the University of Kentucky College of Pharmacy and Dr. Bret Smith of the University of Kentucky College of Medicine for you input throughout my project and for continuing to challenge me to conduct high quality science.

Finally, I would like to thank my family and friends for your encouragement and unwavering support. Without this, I could not have finished my research or writing this thesis.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

List of Abbreviations

ANOVA	Analysis of Variance
BBB	Blood-Brain Barrier
Cel	Celecoxib
COX-2	Cyclooxygenase-2
cPLA2	Cytosolic Phospholipase A_2
CSF	Cerebrospinal Fluid
DPBS	Dulbecco's Phosphate Buffered Saline
ELISA	Enzyme-Linked Immunosorbent Assay
IACUC	Institutional Animal Care and Use Committee
i.m.	Intramuscular
i.p.	Intraperitoneal
i.v.	Intravascular
LCF	Licofelone
5-LOX	5-Lipoxygenase
MMP	Matrix Metalloproteinase
MW	Molecular Weight
NMDAR	N-methyl-D-aspartate Receptor
PBS	Phosphate Buffered Saline
Pilo	Pilocarpine
PWE	Patients with Epilepsy
P-gp	Permeability glycoprotein
SE	Status Epilepticus
TGF- β	Transforming Growth Factor – β
TJ	Tight Junction
TR	Texas Red™
ZC	Zileuton and Celecoxib
Zil	Zileuton

1. Introduction

1.1 Epilepsy Background

Epilepsy is the 4th most prevalent neurologic disorder after migraine, stroke, and Alzheimer's disease: affecting 50 million people worldwide and 3.4 million people in the USA have epilepsy and at least 30% of all epilepsy patients are refractory to drug therapy.[2-5] The prevalence of epilepsy increased since the previous estimate of 3 million people which is thought to be due to population growth.[2] One factor which may influence the incidence of epilepsy in the near future is the expanded definition of epilepsy established by the International League Against Epilepsy (ILAE).[6] According to this definition, diagnosis of epilepsy requires one of the following: 1) two unprovoked seizures occurring at least 24 hours apart; 2) one unprovoked seizure with a high risk of recurrence; 3) diagnosis of an epilepsy syndrome. The latter two characteristics were added to address a patient's risk of future seizures and help epileptologists in treatment decisions.

Living with the threat of future seizures places a significant burden on daily life of epilepsy patients. Only Alzheimer's disease, stroke, and migraines have higher disability-adjusted life years (WHO Neurological Disorders: Public Health Challenges Report). In addition to disease burden, patients with epilepsy (PWE) are frequently afflicted with comorbid neurobehavioral conditions such as depression, suicidal ideation or attempt, cognitive impairment and drug abuse in addition to recurrent seizures.[7-10]

1.2 Status Epilepticus Definition, Treatment, and Outcomes

Status epilepticus (SE) was most recently redefined by the ILAE as *"a condition resulting either from the failure of the mechanisms responsible for seizure termination or from the initiation of mechanisms which lead to abnormally prolonged seizures (after time point t_1). SE is a condition that can have long-term consequences (after time point t_2) …"*[11] For generalized convulsive SE, seizures lasting > 5 minutes or recurrent seizure activity without recovery is used to operationally define SE.[12]

Benzodiazepines are considered first line for initial pharmacologic treatment of SE and the specific agent and route of administration is determined based on factors such as the availability and timeliness of IV access and the age of the patient.[12-15] For instance, while i.v. lorazepam may provide the shortest latency to seizure cessation, i.m. midazolam would be preferred for patients without prior i.v. access.[16] If a patient is still seizing after 20 minutes of benzodiazepine administration, a 2nd line agent such as i.v. fosphenytoin, i.v. valproic acid, or i.v. levetiracetam is administered. However, no trial has directly compared these three treatments head-to-head to determine which is best. The Established Status Epilepticus Treatment Trial (ESETT) is a triple blind randomized clinical trial currently underway to assess which of these three treatments is most effective in ending benzodiazepine-refractory SE.[17,18] After a patient does not respond to a second anti-seizure drug, the SE is termed refractory and treatment varies widely by institution, but most commonly includes a continues i.v. infusion of an anesthetic agent such as midazolam, propofol, or a barbiturate.[19,20] SE has far reaching outcomes for patients including later progression to epilepsy, cognitive impairment, and death. Mortality during or after SE remains a severe, but common outcome with estimates of 30% mortality in adults.[13,21] In a meta-analysis by Lv and colleagues, the overall mortality rate for SE was considerably lower than 30%, but the authors noted a 33% mortality rate in studies and included analysis of RSE. Additionally, they found that etiology and age can also be a predictor of mortality.[22] Pediatric SE patients have a much lower risk of death (3-5%) compared to elderly patients (25-31%).[22-26]

One of the most significant outcomes of seizures, and especially SE, is the risk of recurrent seizures. The phenomenon that "seizures beget seizures" has been observed since the 1800's when Sir William Gowers noted that patients who have at least two seizures are more likely to have future seizures and as the time between seizure events decreases, the less likely it becomes for a patient to stop having seizures.[27] Indeed, the risk of experiencing further seizures in epilepsy patients and when to diagnose a patient with epilepsy after a first seizure is currently an area of research and debate.[28-30] After SE, the risk of developing further seizures is considerable with about 40% of adults developing epilepsy within 10 years after an SE event.[31] In pediatric populations, the reported risk of developing epilepsy is highly variable depending on study design and duration of follow up.[24] The incidence of pediatric patients developing epilepsy with SE as the second seizure event for diagnosis ranges from 10.5-82% over 10 years.[24,32,33] The clinical observation that seizures beget seizures is well documented, but the pathophysiology behind this phenomenon is still poorly understood and may be related to changes to the blood-brain barrier.

1.3 Blood-Brain Barrier Background

The blood-brain barrier is composed of a continuous capillary network of endothelial cells and serves as a selective interface between the cerebrovasculature (**Figure 1**) and the brain parenchyma. Paul Ehrlich first made the observation that the CNS was different than other organ systems in that the brain and spinal cord were not stained by intravenous (i.v.) injected dyes, but the term blood-brain barrier was not coined until later by Max Lewandowski when he noted that the brain was sheltered of pharmacologic effects from i.v. administration of bile acids.[34,35] Ehrlich's student, Edwin Goldman, later demonstrated that intraventricular administration of Trypan blue stained only brain tissue: concluding that the brain was a protected compartment from the blood.[36]

This early work was expanded in a significant way with the advent of the electron microscope. With this new technology, Reese, Karnovsky, and Brightman were able to show that tight junctions in brain capillaries exclude paracellular transport of tracer molecules such as horse radish peroxidase (MW 40,000 Da) and lanthanum (MW 139) from passing into the brain parenchyma.[37,38] Tight junctions are complex protein structures that include transmembrane proteins such as zona occludens, claudins, and junction adhesion molecules which seal the space between endothelial cells to form an anatomical barrier which prevents sanguineous materials from entering the brain and vice versa.[39,40] Dysfunction and leakage of the blood-brain barrier has been implicated in pathologies of numerous disease states such as stroke, Alzheimer's disease, amyotrophic lateral sclerosis, multiple sclerosis, and epilepsy.[41-46]

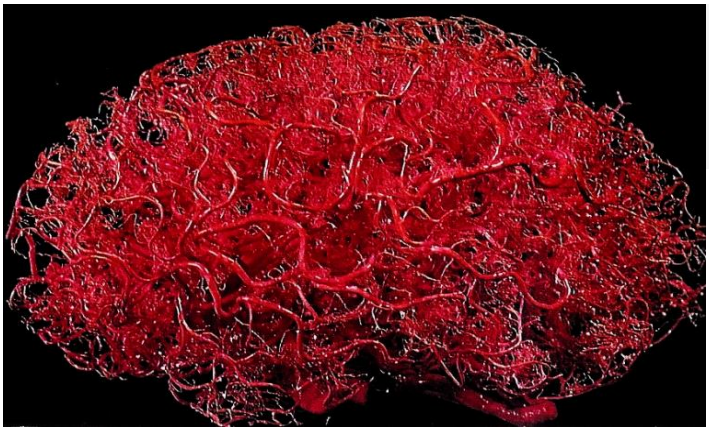

Figure 1: Latex Mold of Cerebrovasculature. Adopted for use with license agreement from Oxford University Press.[1]

1.4 Clinical Association Between Blood-Brain Barrier Opening and Seizures

There is a variety of clinical evidence to support the association between seizures and blood-brain barrier opening. The blood-brain barrier normally excludes sanguineous proteins such as albumin from the brain. However, albumin extravasation has been seen by immunohistochemistry in resected tissue from temporal lobe epilepsy patients with complex partial seizures and patients with secondary generalized seizures.[47] Of note, profoundly more extravasation was observed in PWEs who died during SE. In patients with primary CNS lymphomas, one developing treatment option is to undergo blood-brain barrier disruption with hyperosmotic mannitol.[48] Focal seizures are a common side effect of this treatment with 33.6% of patients having seizure activity despite prophylactic treatment with anti-seizure drugs.[49] Sera from these patients also have significantly elevated S100β levels. S100β is also being investigated as a marker of blood-brain barrier leakage and as a prognostic marker after sports-related concussions and traumatic brain injury.[50-54] Clinical seizures are seen in 12% of patients with severe traumatic brain injury and early seizure prophylaxis with phenytoin or levetiracetum is common.[55] There is a clear association between breakdown of the blood-brain barrier and seizures, but the mechanisms which regulate blood-brain barrier leakage and treatment strategies are still in need of investigation.

1.5 Mechanism of Blood-Brain Barrier Leakage in Epilepsy

Data from animal experiments have shown that blood-brain barrier opening can lead to epileptogenesis.[56,57] Additionally, findings from studies show blood-brain barrier leakage after the development of seizures.[48,58] These observations support the clinically held canon that “seizures beget seizures” and the underlying molecular mechanisms and pharmacologic targets of blood-brain barrier leakage in epilepsy are central to this thesis.

Glutamate is the primary excitatory neurotransmitter in the brain and is released in large amounts during seizure activity.[59,60] After seizure-induced release, glutamate causes changes at the blood-brain barrier such as increased P-glycoprotein (P-gp) expression.[61] This effect is mediated by glutamate through a signaling pathway involving N-methyl-D-aspartate receptor (NMDAR) on brain capillaries, arachidonic acid, and the cyclooxygenase-2 (COX-2) enzyme.[62,63]

Exposing isolated rat brain capillaries *ex vivo* to glutamate increased MMP protein expression and activity, decreased TJ protein expression, and increased capillary leakage.[64] Similar changes were observed in a status epilepticus rat model. These effects were abolished with inhibitors of cytosolic phospholipase A2 (cPLA2). Additionally, these effects were absent when brain capillaries isolated from cPLA2 knock-out mice were exposed to glutamate.[64] Given what is known about the connection between P-gp and the glutamate-arachidonic acid-COX-2 pathway, taken together with unpublished cPLA2 data, we conclude blood-brain barrier leakage to be a result of the same pathway. Further unpublished *ex vivo* data from this laboratory suggest that upregulation of MMPs, degradation of TJs, and barrier leakage are also mediated by the NMDAR, cPLA2, arachidonic acid, and COX-2. However, preliminary data also suggest inhibition of COX-2 alone is insufficient to prevent these changes. Indeed, inhibition of 5-lipoxygenase (5-LOX) in addition to COX-2 inhibition ameliorated the upregulation of MMPs, TJ degradation, and barrier leakage suggesting that both enzymes are involved in causing barrier leakage. Thus, we propose a positive feedback loop in which glutamate released during seizures acts on capillary NMDA receptors to cause blood-brain barrier disruption through cPLA2, COX-2 and 5-LOX leading to increased MMP expression, degradation of TJs and increased barrier leakage which increases the likelihood of further seizures (**Figure 2**). While the mechanisms behind barrier leakage have been thoroughly investigated in isolated brain capillaries *ex vivo* by our laboratory, the next logical step is

to confirm biological relevance of this pathway by pharmacologic prevention of barrier leakage *in vivo*. We hypothesize that seizures trigger LOX and COX mediated blood-brain barrier leakage and that dual LOX/COX inhibition prevents barrier leakage in an *in vivo* status epilepticus model.

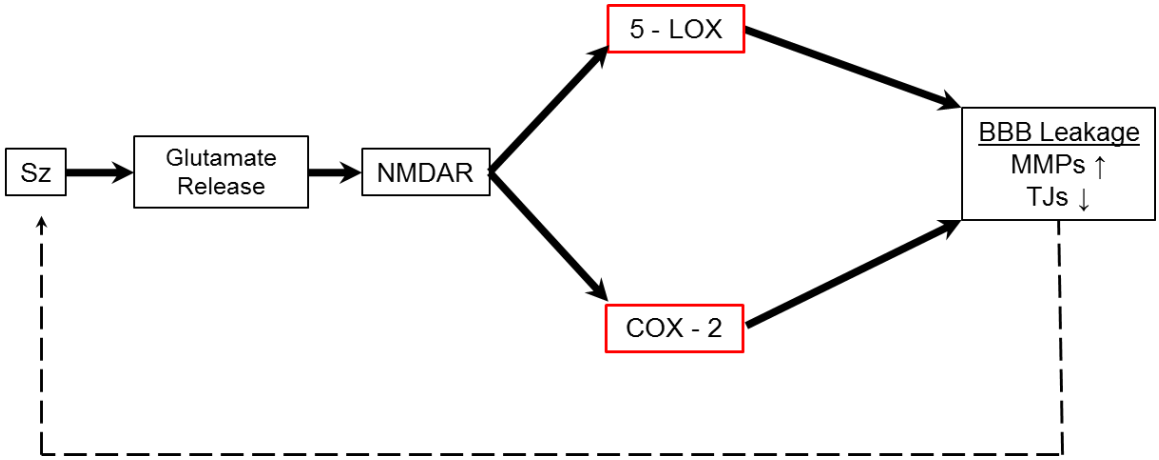

Figure 2: Proposed Positive Feedback Loop for Epileptogenesis. A positive feedback loop in which glutamate released during seizures propagates further seizures by glutamate acting on capillary NMDA receptors to cause blood-brain barrier disruption through COX-2 and 5-LOX which increase MMP expression, degradation of TJs to increase barrier leakage which increases the likelihood of further seizures.

2. Methods

2.1 Chemicals, Equipment and Materials

Table 2.1.1: Chemicals

Item	**Use**	**Manufacturer**	**Item Number**	Lot(s)
Dulbecco's Phosphate Buffered Saline w/ Ca^{++} and Mg^{++}	Capillary Isolation	GE Healthcare Life Sciences (Logan, UT)	SH30264.01	AC10538277, AB216610
Glucose	Capillary Isolation	Millipore Sigma, Burlingon, MA, USA	G7528-250G	037K01901
Sodium Pyruvate	Capillary Isolation	Millipore Sigma, Burlingon, MA, USA	P2256-25G	SLBP4879V
Ficoll® PM 400	Capillary Isolation	Millipore Sigma, Burlingon, MA, USA	F4375-500G	SLBN3686V
Bovine Serum Albumin 2M	Capillary Isolation	Millipore Sigma, Burlingon, MA, USA	A9647-100G	SLBR6769Vd
Sterile Normal Saline Solution	Injection Formulation	Henry Schein (Dublin, OH)	NDC 50989-641-16	B1704082
Dextrose Solution 50%	Injection Formulation	Henry Schein (Dublin, OH)	002460	A1610091

Table 2.1.1 (Continued)

Licofelone	Injection Formulation	Santa-Cruz, Dallas, TX; Toronto Research Chemicals	SC-207826; L397730	A1912, J1317; 7-MAK-90-1
Zileuton	Injection Formulation	Cayman Chemicals,	10006967	0490336-18
Celecoxib	Injection Formulation	Selleckchem, Houston, TX	S1261	S126104
Scopolamine Methylbromide	Injection Formulation	Millipore Sigma, Burlingon, MA, USA	S8502-1G	SLBL9646V
Lithium Chloride	Injection Formulation	Millipore Sigma, Burlingon, MA, USA	L4408-100G	SLBP9949V
Pilocarpine HCL	Injection Formulation	Millipore Sigma, Burlingon, MA, USA	P6503-10G	MKBZ8075V, MKBW5175V
Tween 80	Injection Formulation	Millipore Sigma, Burlingon, MA, USA	P1754-500ML	MKBR2340V
PEG 200	Injection Formulation	Millipore Sigma, Burlingon, MA, USA	P3015-250G	MKBR7821V
Texas Red™	TR Leakage Assay	Sigma, St. Louis, MO, USA	S3388	068K3799V
D-Mannitol	TR Leakage Assay	Sigma, St. Louis, MO, USA	M4125	099K0007
Complete Protease Inhibitor Tablets	Membrane Fraction Isolation	Roche Diagnostics GmBH, Mannheim, Germany	11697498 001	10659100
1X Phosphate Buffered Saline (PBS) w/o Ca2+ and Mg2+	Membrane Fraction Isolation	GE Healthcare Life Sciences (Logan, UT)	SH30256. 01	AC10237763, AC10260338
CellLytic MT Cell Lysis Reagent	Membrane Fraction Isolation	Millipore Sigma, Burlingon, MA, USA		
NaOH	PH Adjustment	Fluka Analytical	71474-1L	BCBM3206V
HCl 1N	PH Adjustment	Fisher Chemical	SA48-500	161478
Human S100β ELISA Kit	S100β ELISA	Millipore Sigma, Burlingon, MA, USA	EZHS100 B-33K	2985955,
Protease Inhibitor	S100β ELISA	Millipore Sigma, Burlingon, MA, USA	539131-10VL	D00173716
Baby Food	Animal Recovery	Beech-Nut Nutrition Co., Amsterdam, NY, USA	Apple and Pear Flavored	
Rodent critical Care Diet – Professional Line	Animal Recovery	Oxbow Animal Health	N/a	163546r

Table 2.1.2: Equipment and Key Materials

Item	Manufacturer	Use
Sorvall™ Legend™ XTR Tabletop centrifuge	Sorvall, ThermoFisher Scientific,	Capillary Isolation
50 ml Potter-Elvehjem tissue grinder (Clearance 150-230 um)	Thomas Scientific, Swedesboro, NJ, USA	Capillary Isolation
15 ml Dounce Homogenizer (Clearance: 80-130 uM)	VWR, Radnor, PA, USA	Capillary Isolation
S6 E Greenough Stereomicroscope	Leica Microsystems, Wetzlar, Germany	Capillary Isolation
Dumont #5 Forceps	Fine Science Tools, Heidelberg, Germany	Capillary Isolation
RZR 2102 Control Electronic Stirrer	Heidolph, Schwabach, Germany	Capillary Isolation
300 um filter mesh	Spectrum Laboratories	Capillary Isolation
pluristrainer® Filters (pore size 30 um)	PluriSelect	Capillary Isolation
15 ml Falcon® tube	Corning™, Corning, NY, USA	Capillary Isolation
50 ml Falcon® tube	Corning™, Corning, NY, USA	Capillary Isolation
Axiovert 25 Inverted Phase Contrast Microscope	Zeiss, Oberkochen, Germany	Capillary Isolation
Fiberlite™ F15-8x50cy 25 degree fixed angle rotor	ThermoFisher Scientific, Waltham, MA, USA	Capillary Isolation
TX-750 Swinging Bucket Rotor	ThermoFisher Scientific, Waltham, MA, USA	Capillary Isolation
Millex® GV 0.22 um filter unit	Millpore Sigma	Capillary Isolation
50 ml Centrifugation tube	ThermoFisher Scientific, Waltham, MA, USA	Capillary Isolation
accuSpin Micro 17R fixed angle microcentrifuge	Fischer Scientific, Pittsburgh, PA	ELISA
Synergy H1 Plate Reader	BioTek, Winooski, VT, USA	ELISA
Gen5™ Software	BioTek, Winooski, VT, USA	ELISA
Polytron™ PT 2500 E Ultraspeed Homogenizer	Kinematica, Luzern, Switzerland	Membrane Fraction Isolation
3.5 ml Ultracentrifuge tubes	Beckman Coulter, Brea, CA, USA	Membrane Fraction Isolation

Table 2.1.2 (Continued)

TLA 100.3 Fixed angle 30 degree Ultracentrifuge Rotor	Beckman Coulter, Brea, CA, USA	Membrane Fraction Isolation
Optima TLX Ultracentrifuge	Beckman Coulter, Brea, CA, USA	Membrane Fraction Isolation
2 mm micro stir bar	VWR International, Radnor, PA	Membrane Fraction Isolation
Marathon 3200R swing-head centrifuge	Fischer Scientific, Pittsburgh, PA	Rat Serum Isolation
3.5 ml SST Vacutainer	Becton Dickinson, Franklin Lakes, NJ	Rat Serum Isolation
Smart2Pure DI water filter	Thermo Scientific	ELISA
Leica TCS SP5 Confocal Microscope	Leica Instruments, Ewtzlar, Germany	TR Leakage Assay
Image J Software v. 1.48v	Wayne Rasband, NIH, USA	Data Analysis
GraphPad Prism 7	GraphPad, San Diego, CA, USA	Data Analysis

2.2 Buffers

Table 2.2.1: Phosphate Buffered Saline without Ca^{++} or Mg^{++}

Additive	mg/L	mmol/L
KH_2PO_4 (Anhydrous)	144	1.06
NaCl	9000	154.0041
Na_2HPO_4	795	5.6002

Table 2.2.2: Isolation Buffer: DPBS with Ca^{++}, Mg^{++}, Glucose, and Pyruvate (pH 7.4)

Additive	mg/L	mmol/L
CaCl	100	0.9
KCl	200	2.7
KH_2PO_4 (Anhydrous)	200	1.47
$MgCl\text{-}6H_2O$	100	0.49
NaCl	8000	136.9
Na_2HPO_4	1150	8.1
D-Glucose	900.8	5
Sodium Pyruvate	110	1

Table 2.2.3: BSA Buffer: 1% BSA in DPBS with Ca^{++}, Mg^{++}, Glucose, and Pyruvate (pH 7.4)

Additive	mg/L	mmol/L
CaCl	100	0.9
KCl	200	2.7
KH_2PO_4 (Anhydrous)	200	1.47
$MgCl\text{-}6H_2O$	100	0.49
NaCl	8000	136.9
Na_2HPO_4	1150	8.1
D-Glucose	900.8	5
Sodium Pyruvate	110	1
Bovine Serum Albumin	10000	

Table 2.2.4: Lysis Buffer

Additive	Quantity Required	Concentration
50x Complete Protease Inhibitor Solution	500 µl	9.1% (4.55x)
CellLytic™ MT Lysis Reagent	5 ml	90.9%
Total	5.5 ml	

Table 2.2.5: LPC Buffer

Additive	Quantity Required	Concentration
Complete Protease Inhibitor	1 tablet	10x
CellLytic™ MT Lysis Reagent	5 ml	50%
PBS w/o Ca^{++} or Mg^{++}	5 ml	50%
Total	10 ml	

2.3 Injection Formulations

Table 2.3.1: Vehicle

Additive	Quantity Required	% Volume
PEG 200	5 ml	5%
Tween 80	1 ml	1%
Normal Sodium Saline	94 ml	94%
Total	100 ml	

Table 2.3.2: Licofelone

Additive	Quantity Required	Concentration
LCF	40 or 80 mg	2 or 4 mg/ml
PEG 200	1 ml	5%
Tween 80	200 µl	1%
Normal Sodium Saline	18.8 ml	94%
Total	20 ml	

Table 2.3.3: Zileuton and Celecoxib

Additive	Quantity Required	Concentration
Zileuton	40 mg	2 mg/ml
Celexoxib	80 mg	4 mg/ml
PEG 200	1 ml	5%
Tween 80	200 µl	1%
Normal Sodium Saline	18.8 ml	94%
Total	20 ml	

2.4 Animal Housing and Handling

Female Wistar rats (10 weeks of age, 180-220 g) were purchased from Charles River Laboratories (Portage, MI), housed under standard conditions of 23º C, 35% relative humidity, 12-hour light/dark cycle and allowed access to water and standard rodent diet *ad libitum* (Harlan Tecklad Chow 2918, Harlan Laboratories Inc., Indianapolis, IN,). Each rat was handled for 1-2 minutes daily for a minimum of 3 days prior to any experiment. All procedures were approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Kentucky protocol #2014-1234 (PI: Bauer).

2.5 Pilocarpine Status Epilepticus Induction in Rats

SE was induced using a modified lithium chloride and pilocarpine model.[65-67] Rats were pre-treated with lithium chloride (127 mg/kg, i.p.) 12 to 15 hours before the first pilocarpine dose and with scopolamine methylbromide (1 mg/kg, i.p.) 30 minutes before the first pilocarpine administration. SE was induced in rats by administration of the muscarinic agonist pilocarpine HCl every 20 minutes. SE was induced by an i.p. bolus of 30 mg/kg pilocarpine was given for the first dose followed by 10 mg/kg i.p doses thereafter until SE was achieved. All seizures during the induction of SE were scored based on Racine's scale[68] and SE was defined by continuous tonic-clonic seizures or intermittent class 4 and 5 seizures without recovery of normal conscious behavior in between. SE was terminated after 90 minutes by i.p. administration of 10 mg/kg diazepam every 20 minutes until cessation of visible seizures. Rats that received pilocarpine but did not enter SE served as a control group (pilocarpine control, pilo) to ensure that observations made of the SE group are due to seizure activity during 90 minutes of SE and not pharmacologic effects of pilocarpine. The negative control rats received LiCl, scopolamine methylbromide, diazepam, and normal saline in place of pilocarpine.

After SE termination with diazepam, rats were given 3-5 ml 5% dextrose in normal saline (D5NS) by s.c. administration. For experiments in which rats were euthanized 24 or 48 hours after SE cessation, D5NS was also administered in the evening after the induction and the following morning. Rats were closely monitored until euthanasia and additional doses of diazepam were given when seizure activity was observed. Recovering rats were fed apple sauce, rodent intensive care diet, and softened rodent chow until the animals were able to resume normal feeding behavior.

2.6 Time Course Design

Five SE inductions were conducted as previously described and serum was collected at three time points: immediately following SE termination (n = 2 experiments), 24 hours after SE termination (n = 1 experiment), and 48 hours after SE (n = 2 experiments) (**Figure 3**). These samples were used to determine at which time point S100β would be most consistently elevated.

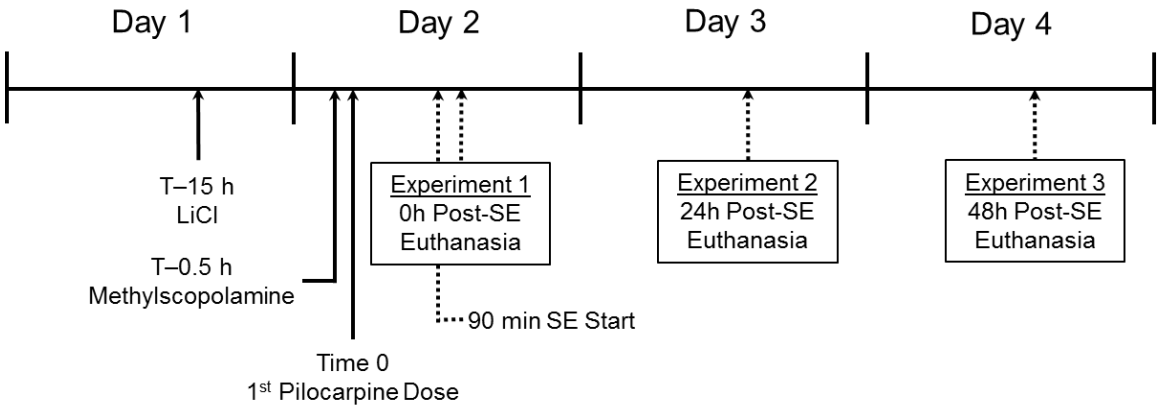

Figure 3: Blood-Brain Barrier Leakage Time Course. At three time points, serum samples were collected to measure S100β concentrations after SE.

2.7 Licofelone Study Design

Rats underwent SE induction as described above. In addition, rats were dosed i.p. with either vehicle or the dual LOX and COX inhibitor licofelone (LCF).[69] This study was divided into two experiments using 5 mg/kg and 10 mg/kg licofelone.[70] LCF or vehicle was given every 12 hours starting 2 days before the induction (**Figure 4**). In each experiment, this produced 4 groups of animals, in addition to a control group, defined by whether they received vehicle or LCF and whether or not they developed SE (Pilo-Veh, Pilo-LCF, SE-Veh, SE-LCF).

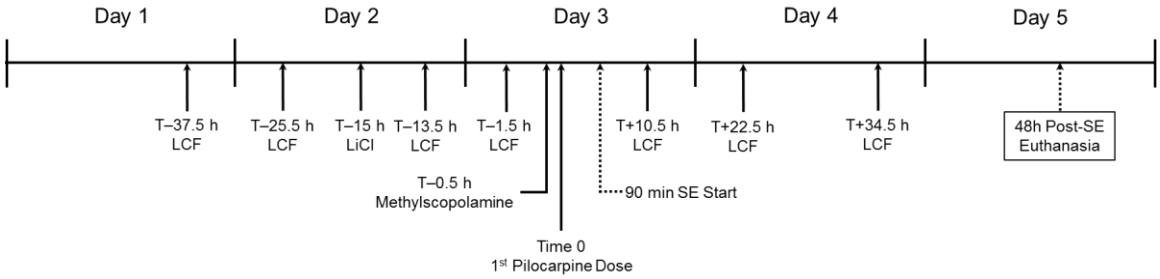

Figure 4. Timeline for Licofelone Experiments. The timeline is centered on the time of the first pilocarpine injection with interventions labeled at Time 0 ± hours. Sera were collected 48 hours after SE termination and used to measure S100β levels.

2.8 Zileuton & Celecoxib Study Design

Rats underwent SE induction as described above. In addition, rats were dosed i.p. with either vehicle or the 5-LOX inhibitor zileuton (5 mg/kg) and the COX-2 inhibitor celecoxib (10 mg/kg) (ZC). ZC or vehicle was given every 12 hours starting 2 days before the induction. (**Figure 5**). In each experiment, this produced 4 groups of animals, in addition to a control group, defined by whether they received vehicle or LCF and whether or not they developed SE (Pilo-Veh, Pilo-ZC, SE-Veh, SE-ZC).

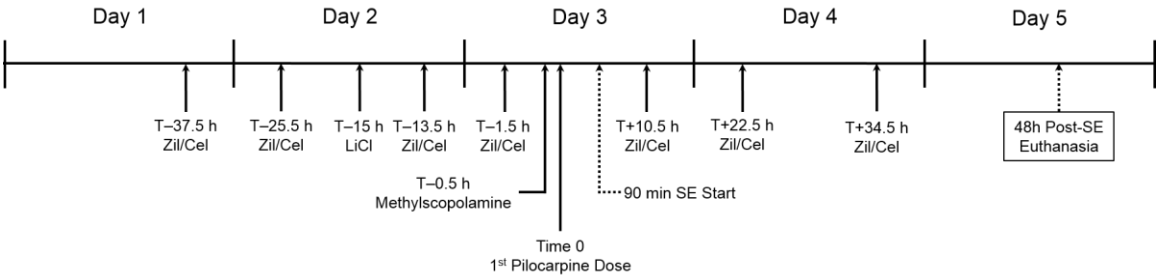

Figure 5. Timeline for Zileuton + Celecoxib Experiments. The timeline is centered on the time of the first pilocarpine injection with interventions labeled at Time 0 ± hours. Sera were collected 48 hours after SE termination and used to measure S100β levels

2.9 Rat Serum Isolation

Rats were euthanized by CO_2 inhalation 0, 24, or 48 hours after SE termination. The thoracic cavity was opened and blood was drawn via right ventricular heart puncture. This blood was collected in a 3.5 ml SST vacutainer carefully inverted 5 times and incubated at room temperature for 30 minutes. The tubes were centrifuged at 1,300 g for 10 minutes at 4 ºC in a swing-head centrifuge. Serum samples were taken as aliquots of the supernatant and kept for short term storage at -20 ºC until use.

2.10 S100β ELISA

ELISA reagents and standards as well as serum samples were prepared and plated as recommended in the manufacturer's protocol (Kit # EZHS100B-33K, MilliporeSigma, Burlington, MA). The reaction product was measured by absorbance at 450 nm using a Synergy H1 plate reader and corrected with a reference absorbance at 590 nm. The absorbance was compared against a standard curve to calculate the concentration of S100β.

2.11 Capillary Isolation

Rats were euthanized by CO_2 inhalation and decapitated. Brains were collected in ice-cold isolation buffer. Using a stereo microscope, the meninges, choroid plexus, brain stem, and white matter were removed with Dumont #5 forceps and the remaining cortical tissue was minced with a scalpel. This tissue underwent two homogenization steps. In the first homogenization, the minced tissue and 3 ml of isolation buffer per rat brain was added to a 50 ml Potter-Elvehjem homogenizer (clearance: 150-230 µm). 100 up-and-down strokes were used to homogenize the tissue using an overhead stirrer set to 50 rpm. Then, the homogenate was transferred to a Dounce homogenizer (clearance: 25-75 µm) and homogenized further with 20 strokes. The homogenate was then divided equally into two centrifugation tubes and 2.5 ml of 30% Ficoll® per rat brain plus 0.5 ml of isolation buffer per rat brain were added to each tube. The homogenates were separated by density

centrifugation at 5,800 g for 15 min at 4 ºC in a 45º fixed-angle rotor. The supernatant was discarded and the pellet containing capillaries, red blood cells, and other cellular debris was resuspended in BSA buffer and filtered through a 300 µm mesh to remove larger vessels and brain debris. The filtrate was passed through a 30 µm pluriStrainer® after which the majority of capillaries remained on top of the filter.[64] The filtrate was passed through another 30 µm pluriStrainer® to minimize capillary loss. The capillaries were washed off of each filter by inverting it over a 50 ml Falcon tube and rinsing it with 50 ml BSA buffer. These tubes were centrifuged at 1,500 g for 3 min at 4 ºC and the pellet (**Figure 6**) was resuspended in isolation buffer and transferred to a 15 ml conical tube and filled to 15 ml with isolation buffer. After another centrifugation at 1,500 g for 3 min at 4 ºC and washing, capillaries were either used for *ex vivo* Texas Red™ leakage assays or for capillary membrane isolations.

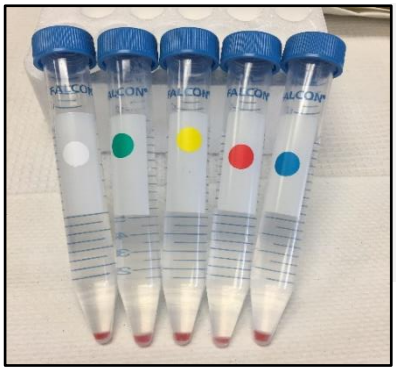

Figure 6: Isolated rat brain capillary pellets. A representative image of the capillary pellet size. These capillaries are resuspended for confocal microscopy and for isolation of capillary membrane fractions.

2.12 Capillary Membrane Fraction Isolation

Capillaries suspended in isolation buffer were centrifuged at 4,700 g for 1 min at 4 ºC and the supernatant was discarded. The pellets were resuspended in lysis buffer and transferred into ultracentrifuge tubes. Capillaries were homogenized for 3 min 20 seconds using an ultraspeed homogenizer at 30,000 RPM. The crude membrane fraction was isolated by differential centrifugation. First, the homogenates were centrifuged at 30,000 RPM (38,203 x g) for 30 min at 4 ºC. The supernatant containing the cellular membrane fraction was transferred in a new set of centrifugation tubes using a Pasteur pipet and centrifuged again at 95,000 RPM (383,093 x g) for 2 hours at 4 ºC; the remaining pellet containing cell debris and other cellular organelles was discarded. The resulting pellets (**Figure 7,** crude membrane fraction) were resuspended in LPC buffer, transferred to microcentrifuge tubes and stored at -80 ºC.

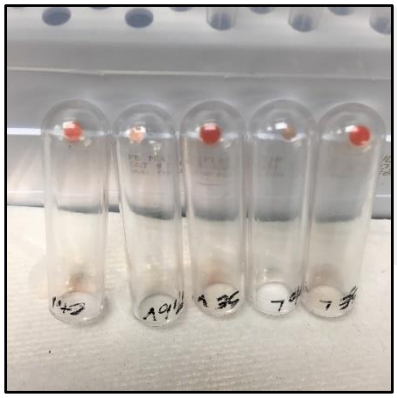

Figure 7: Isolated membrane fraction of rat brain capillaries. Representative image of membrane fractions pellets. These isolates will be used to determine capillary membrane protein concentrations.

2.13 Texas Red™ Leakage Assay

Texas Red™ (TR) leakage assays were performed as previously described.[71,72] TR is a fluorescent organic anion (MW 625 Da, excitation wavelength 543 nm, emission wavelength 615 nm) and is actively transported into the lumen of brain capillaries. When isolated brain capillaries are incubated in solution with TR for 1 hour, the fluorescent molecule accumulates in the lumen of the capillaries and can be quantified using confocal microscopy (**Figure 8**) and image analysis software. Confocal microscope setting were as follows: 63.0x1.2 D-water UV objective, pinhole: 111.4 µm, zoom: 4, image format: 512x512, scanning speed: 400 Hz, line average: 1, frame average: 3, laser: 543 HeNe. If the brain capillaries are leaky, TR leaks out of the lumen, and thus, luminal TR fluorescence decreases over time. Assuming first order elimination kinetics, this information can be used to calculate a leakage rate using non-linear regression analysis.

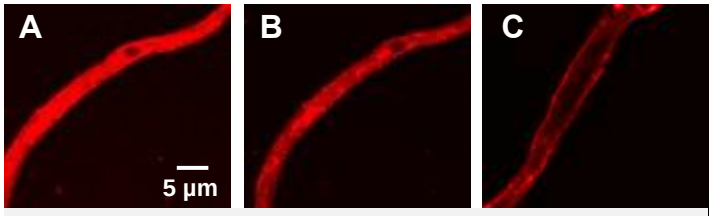

Figure 8: TR Capillary Leakage

A: Control capillary loaded with 2 µM Texas Red at time point 0 min

B: Control capillary after removal of Texas Red at time point 60 min

C: Capillary treated with Mannitol with after removal of Texas red at time point 60 min

2.14 Statistical Analysis

Statistical analysis was conducted using GraphPad Prism 7 and results were considered significant when $p<0.05$. Significance reported as: * $p<.05$; ** $p<0.01$; *** $p<0.001$; **** $p<0.0001$. Results for post-SE S100β ELISAs and Texas Red leakage assays were assessed using one-way analysis of variance (ANOVA) with Tukey's test for post-hoc analysis of comparisons between groups.

3. Results

3.1 Detection of Serum S100β by ELISA

Data from previous experiments with isolated capillaries from SE rats suggest that blood-brain barrier leakage occurs 48 hours after SE. To determine if this observation could be corroborated by detection of brain derived protein leaking into the blood, ELISA analysis for serum S100β was performed. S100β is an astrocyte-derived neurotrophic protein not normally found in the blood unless barrier leakage is present.[73] Rat serum samples were analyzed in duplicate by ELISA to determine S100β concentration. A time course was conducted (**Figure 3**) and serum S100β concentrations were measured at 0, 24, and 48 hours after SE. While some SE rats exhibited highly elevated S100β levels immediately after SE compared to control rats or pilocarpine control rats (received pilocarpine but did not develop SE), the effect was inconsistent (**Figure 9**) (SD=189 pg/ml). More specifically, the data seemed to divide into two groups: one group with S100β levels comparable to controls and one group which had distinctly elevated S100β.

At 24 hours after SE, no increase in S100β was observed compared to controls (**Figure 10**). Conversely, serum S100β was reproducibly and significantly increased in SE rats at 48 hours after SE (**Figure 11**). These data corroborate preliminary data showing barrier opening at 48 hours after SE and support serum S100β concentration as a surrogate marker for barrier leakage.

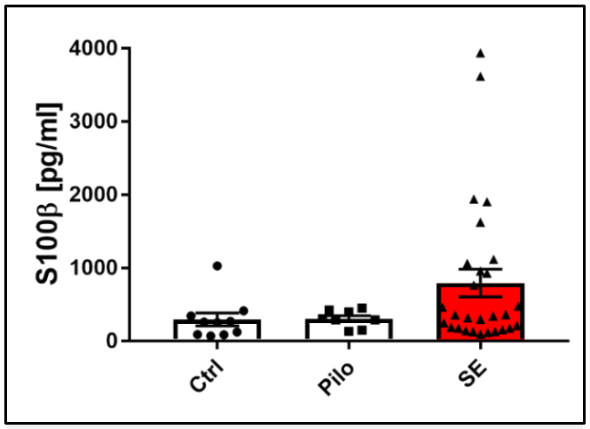

Figure 9. Serum S100β Levels Immediately Following SE. Serum S100β concentrations were non-significantly elevated for SE rats compared to controls and compared to rats that received pilocarpine but did not develop SE. S100β concentrations measured as pg/ml; data are mean ± SEM (n = 2 independent experiments); group sizes: Control n = 10; Pilo n = 8; SE n = 28. Statistical comparison: ns, p=0.139; ANOVA.

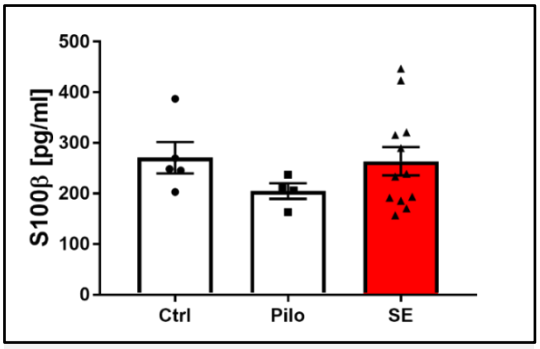

Figure 10. Serum S100β levels 24 Hours After SE. Serum S100β concentrations were similar to those in SE rats compared to controls and compared to rats that received pilocarpine but did not achieve SE. S100β concentrations measured as pg/ml; data are mean ± SEM; Group sizes: Control n = 4; Pilo n = 4; SE n = 12. Statistical comparison: ns, p=0.4316; ANOVA.

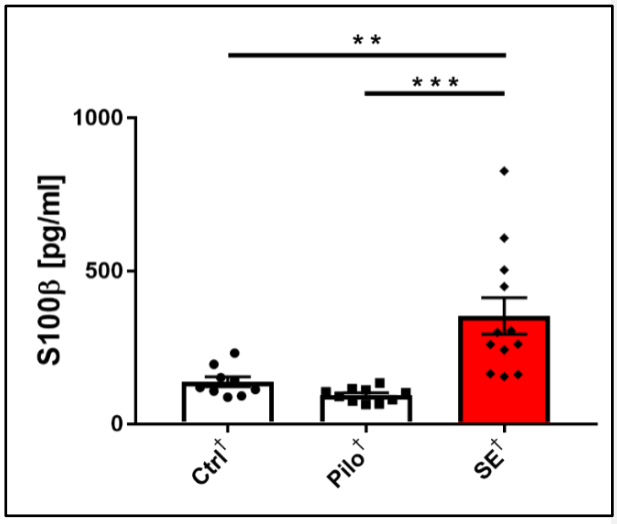

Figure 11. Serum S100β Levels 48 Hours after SE. In pooled data from two 48 hour SE inductions, serum S100β concentrations were significantly elevated for vehicle treated SE rats compared to controls and compared to rats that received pilocarpine but did not achieve SE. S100β concentrations measured as pg/ml; data are mean ± SEM (n=2 independent experiments); group sizes: Control n = 9; Pilo n = 10; SE n = 12. Statistical comparison: **, Control vs SE, $p<0.01$; ***, Pilo vs SE, $p<0.001$; ANOVA with Tukey's post-hoc analysis.

3.2 Prevention of Blood-Brain Barrier Leakage with Licofelone

Since the astrocyte-derived protein S100β is consistently elevated in the serum of SE rats at 48 hours after SE and preliminary *ex vivo* data suggest leakage in brain capillaries is mediated by 5-LOX and COX-2, an *in vivo* study was conducted to determine if inhibition of LOX and COX would prevent S100β elevation after SE. First, rats were dosed with either vehicle or the dual LOX and COX inhibitor licofelone (5 mg/kg, i.p. injection) every 12 hours for 7 doses beginning 2 days before the SE induction. Serum and brain capillaries were isolated from rats after 48 hours. Serum S100β was measured by ELISA (**Figure 12**). Rats from the vehicle and LCF groups that received pilocarpine, but did not develop SE had similar S100β serum levels as the negative controls (61, 73, 75 pg/ml, respectively, $p > 0.99$ for all). Vehicle-dosed rats that developed SE had a 4.8-fold increase in mean S100β serum levels compared to control rats ($p < 0.05$). Similarly, LCF-dosed rats that developed SE had increased mean S100β levels and were significantly greater than controls ($p < 0.05$), but appeared to be divided into two sub-groups: one group with concentrations similar to the SE-Veh group and one with concentrations similar to controls.

To assess if LCF treatment could prevent leakage in capillaries isolated from SE rats, confocal microscopy was used to measure the leakage rate of fluorescent Texas Red™ (**Figure 13**). TR leakage rates for capillaries of the SE-Veh group equaled that of the assay positive control (capillaries incubated in 100 mM, hyperosmotic mannitol). Conversely, isolated capillaries from the vehicle- and LCF-dosed rats that received pilocarpine but did not develop SE had leakage rates that were equal to those in negative control capillaries. Brain capillaries from LCF-treated SE rats had lower leakage rates than those from SE-vehicle rats, but did not decrease to control levels.

Based on these observations, the experiment was repeated with 10 mg/kg LCF (**Figure 14**). Serum S100β was measured by ELISA. Rats from the vehicle and LCF groups that received pilocarpine, but never developed SE, had similar S100β serum concentrations as the negative controls. Vehicle-dosed rats that developed SE had an 8.4-fold increase in S100β concentration compared to controls ($p<0.0001$). In contrast to the previous experiment, LCF-dosed rats that developed SE had significantly lower S100β serum concentrations than SE-Veh rats and were consistent with those of the control and pilocarpine group rats (SE-Veh vs SE-LCF: $p < 0.0001$; Control vs SE-LCF: $p > 0.99$; Pilo-LCF vs SE-LCF: $p=0.92$).

To assess if the increased LCF dose would also prevent leakage in capillaries isolated from SE rats, confocal microscopy was used to measure the leakage rate of fluorescent Texas Red™ (**Figure 15**). TR leakage rates for capillaries of the SE-Veh group () were similar to those of the positive control (SE-Veh: 0.7± 0.23 min^{-1}; 100 mM mannitol: 0.8±0.27 min^{-1}). Conversely, isolated capillaries from the Pilo-Veh, Pilo-LCF and SE-LCF had leakage rates equal to negative control capillaries (Control: 0.2±0.04 min^{-1} Pilo-Veh: 0.2±0.05 min^{-1}; Pilo-LCF: 0.2±0.07 min^{-1}; SE-LCF: 0.2±0.03). Put together with decreased serum S100β in LCF treated SE rats, these data suggest licofelone prevents SE induced blood-brain barrier leakage in a dose-dependent manner (**Figure 16)**.

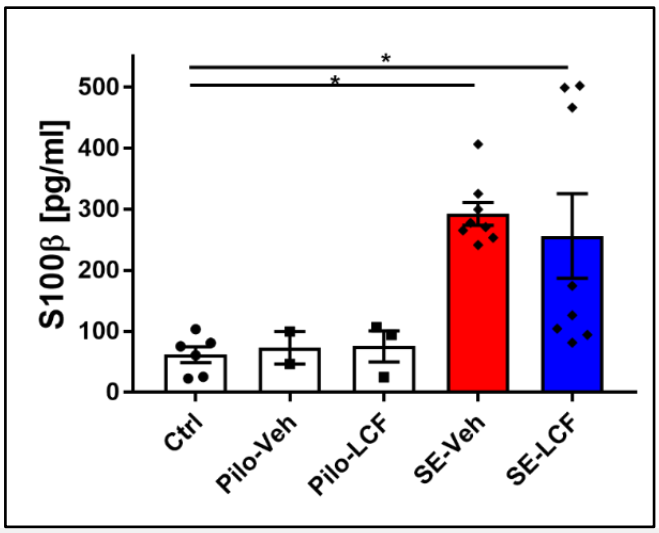

Figure 12: 5 mg/kg Licofelone Does Not Prevent S100β Leakage After SE
Serum S100β concentrations were elevated for vehicle-treated SE rats compared to controls and compared to rats that received pilocarpine but did not achieve SE. SE rats which received LCF also had elevated serum S100β levels on average, but had large variability (SD = 195 pg/ml). S100β concentrations measured as pg/ml; data are mean ± SEM; group sizes: Ctrl n = 6; Pilo-Veh n = 2, Pilo-LCF n = 3, SE-Veh n = 8, SE-LCF n = 8. Statistical comparison: *, Control vs SE-Veh, $p<0.05$; *, Control vs SE-LCF, $p<0.05$; ANOVA with Tukey's post-hoc analysis.

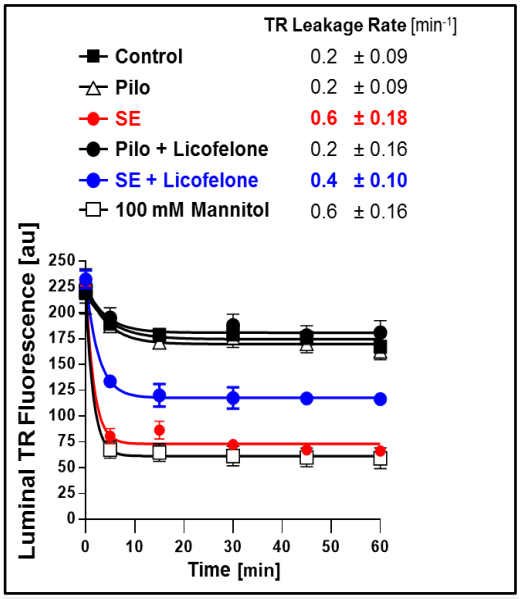

Figure 13: 5 mg/kg Licofelone Decreases Barrier Leakage in Brain Capillaries.
The rate of Texas Red™ leakage from capillaries isolated from LCF-treated SE rats was decreased in comparison to vehicle treated SE rats. However, the leakage rate was not decreased to control levels. Luminal TR measured as arbitrary fluorescence units and first-order efflux rates were calculated by non-linear regression. Efflux rates are shown as mean ± SEM for n = 7 capillaries per time point for each group; group sizes: Ctrl n = 6; Pilo-Veh n = 2, Pilo-LCF n = 3, SE-Veh n = 8, SE-LCF n = 8.

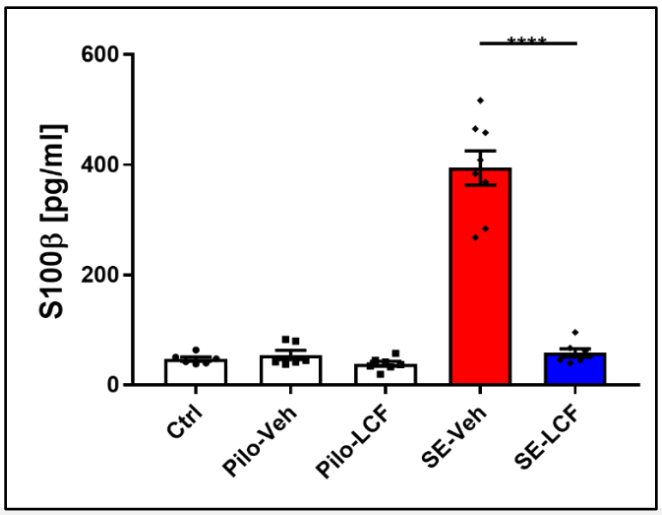

Figure 14. 10 mg/kg licofelone prevents S100β leakage after status epilepticus. Serum S100β concentrations were elevated for vehicle-treated SE rats compared to controls and compared to rats that received pilocarpine but did not develop SE. Rats treated with LCF had significantly lower levels of serum S100β compared to SE rats which received vehicle ($p < 0.0001$). S100β concentrations measured as pg/ml; data are mean ± SEM; group sizes: Ctrl n = 6; Pilo-Veh n = 6, Pilo-LCF n = 7, SE-Veh n = 8, SE-LCF n = 7. Statistical comparison: ****, Control vs SE-Veh, $p<0.0001$; ****, SE-Veh vs SE-LCF; ns, Control vs SE-LCF, $p>0.99$; ANOVA with Tukey's post-hoc analysis.

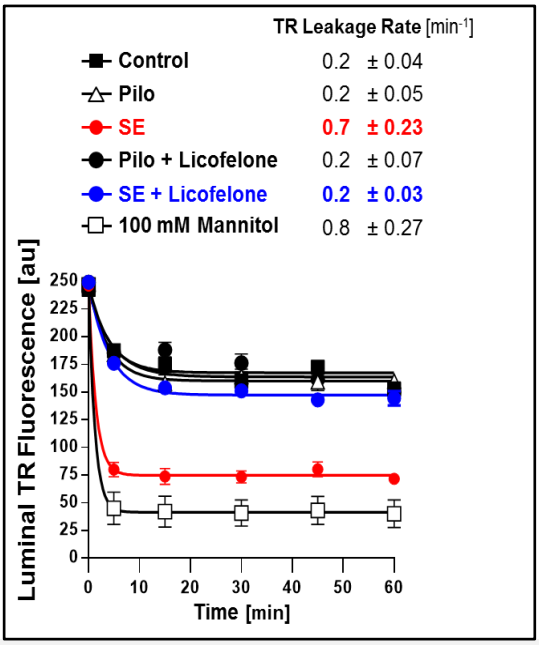

Figure 15: 10 mg/kg Licofelone Decreases Barrier Leakage in Brain Capillaries. The rate of Texas Red™ leakage from capillaries isolated from LCF treated SE rats was decreased to control levels. Luminal TR measured as arbitrary fluorescence units and first-order efflux rates were calculated by non-linear regression. Efflux rates are shown as mean ± SEM for n = 7 capillaries per time point for each group; group sizes: Ctrl 6; Pilo-Veh n = 6, Pilo-LCF n = 7, SE-Veh n = 8, SE-LCF n = 7.

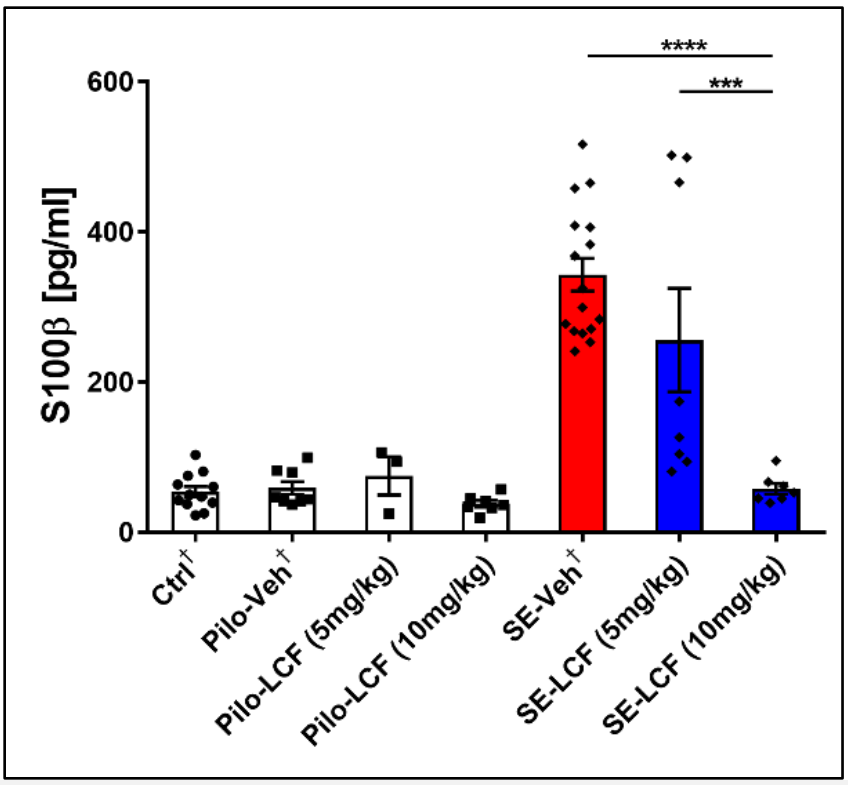

Figure 16: Licofelone Study Dose Response. Licofelone dosing with 10 mg/kg significantly decreased S100β concentrations compared to SE-Veh and SE-LCF (5 mg/kg). Data are pooled (†) from 2 independent experiments for Ctrl, Pilo-Veh, and SE-Veh groups. S100β concentrations measured as pg/ml; data are mean ± SEM; group sizes: Ctrl† n = 12; Pilo-Veh† n = 8, Pilo-LCF (5 mg/kg) n = 3, Pilo-LCF (10 mg/kg) n = 7, SE-Veh† n = 16, SE-LCF (5 mg/kg) n = 8, SE-LCF (10 mg/kg) n =7. Statistical comparison: ****, Control† vs SE-Veh†, p<0.0001; ***, SE-LCF (5 mg/kg) vs SE-LCF (10 mg/kg); ns, Control† vs SE-LCF (10 mg/kg), p>0.99; ANOVA with Tukey's post-hoc analysis.

3.3 Prevention of Blood-Brain Barrier Leakage with Zileuton and Celecoxib

Licofelone has been used in some clinical trials, but it has not been FDA approved.[73,74] Additionally, it lacks COX-2 selectivity and preliminary data suggest this pathway is mediated by COX-2 and not COX-1.[69] Therefore, rats were dosed with either vehicle or the 5-LOX inhibitor zleuton (5 mg/kg, i.p. injection) and the COX-2 inhibitor celecoxib (10 mg/kg, i.p. injection) every 12 hours for 7 doses beginning 2 days before the SE induction. Serum and brain capillaries were isolated from the rats 48 hours after SE. Serum S100β was measured by ELISA (**Figure 17**). Pilo-Veh and Pilo-ZC rats had similar S100β concentrations (51 pg/ml and 49 pg/ml, respectively) as the negative controls (36 pg/ml). Vehicle-dosed rats that developed SE had 11.2-fold increase in mean S100β concentration compared to controls ($p < 0.0001$). SE-ZC rats had significantly lower S100β concentrations than SE-Veh ($p < 0.0001$) and were consistent with that of the control and pilocarpine groups (Control vs SE-ZC: $p>0.99$; Pilo-Veh vs SE-ZC: $p>0.99$; Pilo-ZC vs SE-ZC: $p>0.99$).

To assess if ZC could also prevent leakage in capillaries isolated from SE rats, confocal microscopy was used to measure the leakage rate of fluorescent Texas Red™ (**Figure 18**). TR leakage rates for capillaries of the SE-Veh group (0.6 ± 0.06) were similar to those of the positive control (100 mM mannitol, 0.7 ± 0.17). Conversely, isolated capillaries from the Pilo-Veh and Pilo-ZC rats had leakage rates equal to negative control capillaries (0.2 ± 0.13, 0.2 ± 0.08, and 0.2 ± 0.09, respectively). For SE rats dosed with zileuton and celecoxib, the leakage rate (0.2 ± 0.06) was also equal to control levels. Based on these data, which show decreased serum S100β concentrations and decreased capillary leakage rates in SE rats treated with zileuton and celecoxib, we conclude that inhibition of 5-LOX and COX-2 prevents SE-induced barrier leakage *in vivo*.

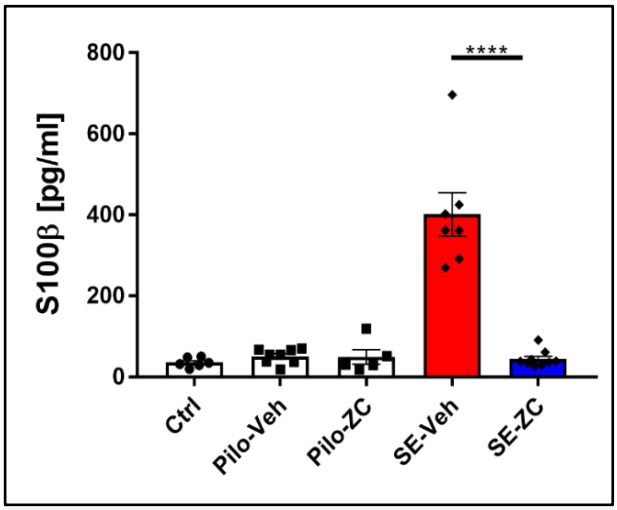

Figure 17: 5 mg/kg Zileuton and 10 mg/kg Celecoxib Prevent S100β Leakage After Status Epilepticus. Serum S100β concentrations were elevated for vehicle treated SE rats compared to controls and compared to rats that received pilocarpine but did not develop SE. Rats treated with ZC had significantly lower levels of serum S100β compared to SE rats which received vehicle. S100β concentrations measured as pg/ml; data are mean ± SEM; Ctrl n = 6; Pilo-Veh n = 8, Pilo-ZC n = 5, SE-Veh n = 7, SE-ZC n = 9. Statistical comparison: ****, Control vs SE-Veh, p<0.0001; ****, SE-Veh vs SE-ZC; ns, Control vs SE-LCF, p>0.99; ANOVA with Tukey's post-hoc analysis.

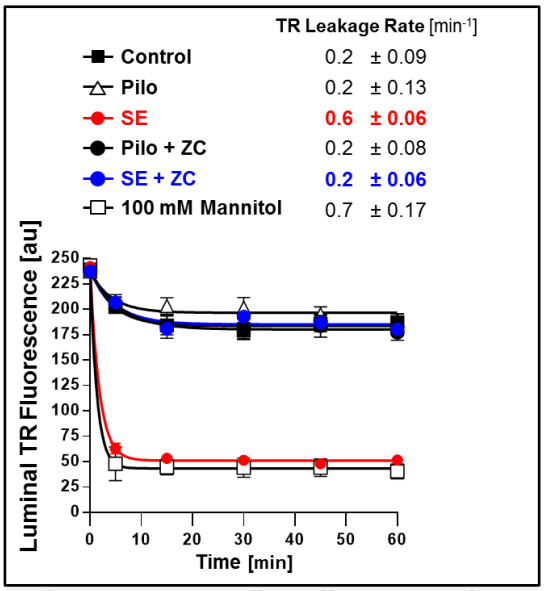

Figure 18: 5 mg/kg Zileuton and 10 mg/kg Celecoxib Decreases Barrier Leakage in Brain Capillaries. The rate of Texas Red™ leakage from capillaries isolated from ZC treated SE rats was decreased to control levels. Luminal TR measured as arbitrary fluorescence units and first-order efflux rates were calculated by non-linear regression. Efflux rates are shown as mean ± SEM for n = 7 capillaries per time point for each group; group sizes: Ctrl n = 6; Pilo-Veh n = 8, Pilo-ZC n = 5, SE-Veh n = 7, SE-ZC n = 9.

4. Discussion

4.1 Summary of Findings

The data in this thesis provide *in vivo* evidence that serum S100β is significantly elevated 48 hours after SE (**Figures 11, 12, 14, 16, and 17**). This finding is reproducible. Additionally, preliminary data were replicated that show capillaries isolated from rats 48 hours post-SE are leakier than those of controls (**Figures 13, 15, and 18**). Taken together, we conclude that S100β is an effective surrogate marker for blood-brain barrier leakage. Knowing that this time point was useful for detecting an acute disruption of blood-brain barrier integrity, rats were treated with the dual LOX and COX inhibitor licofelone. However, no significant reduction in S100β levels or TR leakage rates were found in the SE treatment group compared to SE-Veh rats when licofelone was given at 5 mg/kg (**Figures 12 and 13**). Conversely, 10 mg/kg completely prevented the increased serum S100β and TR leakage seen in SE-Veh rats (**Figures 14 and 15**). These data suggest SE-induced blood-brain barrier leakage can be prevented by licofelone in a dose dependent manner and that this leakage involves LOX and COX (**Figure 16**). Furthermore, inhibition with the FDA-approved inhibitors zileuton and celecoxib show that barrier breakdown is mediated by the 5-LOX and COX-2 isoforms (**Figure 17 and 18**). These data show that inhibiting these enzymes reduced TR leakage to control rates in capillaries isolated from SE rats and also reduced S100β levels to that of controls.

4.2 Findings in Context with Current Literature

In brain tissue, COX-2 mRNA and protein expression increase after seizures with protein expression peaking around 24 hours.[74,75]. COX-2 is an enzyme that generates prostaglandins and conflicting evidence shows that COX-2 inhibition may increase or decrease spontaneous seizure recurrence, mortality, and neuronal damage depending on the model used and whether the inhibitor was given before or after SE induction.[76] COX-2 is also present in brain capillaries, but protein expression remains unchanged when glutamate is applied *ex vivo*.[62,63] Preliminary *ex vivo* data from this group (data not shown) suggest that *ex vivo* brain capillary leakage is prevented by inhibition of LOX and COX together, but not when either of these enzymes is inhibited alone. We conclude that both pathways for metabolizing arachidonic acid are involved in blood-brain barrier leakage. The effects 5-LOX and COX-2 in epilepsy have also been studied by others. Eslami and colleagues recently published work suggesting that licofelone decreases SE incidence.[70] Since we did not induce SE with a single high dose of pilocarpine, but instead used a fractionated multiple-dose method, we could not directly compare SE incidence. However, we did count the number of class 4 and 5 seizures across all SE inductions and found no difference between those treated with vehicle, licofelone, or zileuton with celecoxib (data not shown).

One current hypothesis tying blood-brain barrier leakage to seizure recurrence in pharmacoresistant epilepsy is that extravasation of albumin into the brain decreases free antiepileptic drug concentration.[77,78] If free antiepileptic drug decreases, drug efficacy may decrease as well. One hypothesis for epileptogenesis after blood-brain barrier leakage is that extravasation of albumin causes epileptogenesis through astrocytic transformation.[57,79-84] These studies provide evidence that extravasation of albumin promotes ictogenesis by TGF-β mediated downregulation of inward rectifying potassium channels and glutamate transporters causing impaired buffering of extracellular potassium and glutamate. This is related to our work because currently, there are still knowledge gaps regarding what happens prior to albumin extravasation: the gap between a seizure event and blood-brain barrier leakage. Recent evidence published by our group show that

MMP protein expression and activity is increased after SE and levels of tight junctions decrease.[64] *Ex vivo* preliminary data from this laboratory suggest these changes are mediated by glutamate acting on capillary NMDAR, $cPLA_2$ arachidonic acid production, 5-LOX, and COX-2. As stated above, we hypothesized that seizures trigger LOX and COX mediated blood-brain barrier leakage and that dual LOX/COX inhibition would prevent barrier leakage *in vivo*. The data herein demonstrate barrier leakage associated with seizures is mediated by 5-LOX and COX-2 – as evidenced by serum S100β levels and capillary TR leakage rates – and can be prevented by dual inhibition of these enzymes.

The data presented here are consistent with clinical literature that states seizures are associated with a leaky blood-brain barrier as evidenced by immunohistochemistry and MRI.[47,85] In sera collected from epilepsy patients, S100β concentrations are significantly elevated during seizures compared to sera collected before a seizure event.[86] In the acute seizure model used in this work, serum S100β is profoundly elevated in SE rats supporting it is a translatable model to use for mechanistic studies. With a growing body of literature supporting blood-brain barrier leakage causing seizures, these data would suggest that dual LOX and COX inhibition, such as with zileuton and celecoxib, may be an effective therapy to prevent seizure recurrence.[47,56,87,88] While this study was designed to elucidate the molecular basis for blood-brain barrier opening after SE and identify 5-LOX and COX-2 as therapeutic targets, it was not intended to determine if a "treatment window" exists for these targets. However, Fabene and colleagues demonstrated that administration of adhesion molecule inhibitors after SE decreased polymorphonuclear cell adhesion, prevented barrier leakage after SE, and decreased seizure recurrence in SE.[87] No data exists yet to support a "treatment window" for inhibition of 5-LOX and COX-2. Preliminary data from our laboratory (not shown) suggest that leukotriene B_4, a downstream product of 5-LOX and chemokine for neutrophils, is elevated in plasma from SE rats and epilepsy patients. Additional studies are needed to determine if the LOX pathway is involved in cytokine release and neutrophil recruitment at the blood-brain barrier in epilepsy.[89,90]

4.3 Future Studies

These data provide noteworthy support for inhibition of 5-LOX and COX-2 to prevent of SE induced barrier leakage. However, there are still ways to bolster the validity of these data, the inhibition experiments can be repeated using endpoints such as MRI, cranial window surgery with multiphoton confocal microscopy and a fluorescent vascular marker, *in situ* brain perfusion with a radioactive vascular marker, and brain slice immunohistochemistry for albumin.[57,58,91,92] Showing additional endpoints, with prevention of barrier leakage by inhibiting LOX and COX would enhance the impact of this study. Additional mechanistic studies could also be conducted with inhibitors of other targets that may be downstream of 5-LOX and COX-2. Inhibition of microsomal prostaglandin E_2 synthase 1 is one possible target of interest downstream from COX-2 and inhibitors for this enzyme are currently under development.[93,94] The FDA-approved cysteinyl leukotrieine receptor 1 inhibitor montelukast could be used to inhibit the effects of 5-LOX downstream metabolites. However, this would not account for any actions of dihydroxyl-leukotrienes such as LTB4: a chemokine for neutrophils which have been implicated in blood-brain barrier breakdown and seizure genesis in mice.[87,95] 5-LOX and COX-2 are early actors in the metabolism and action of leukotrienes and prostaglandins and additional mechanistic work may provide additional pharmacologic targets in seizure induced blood-brain barrier leakage.

With evidence to show inhibition of LOX and COX preventing SE-induced barrier opening, the next question should be: does inhibition of LOX and COX prevent seizure recurrence? This would need to be investigated with a chronic epilepsy model (Hartz et

al., (2017). Mol Pharm).[61] If LOX and COX inhibition used as disease modifying therapy added to antiseizure drugs decreases seizure recurrence in a chronic epilepsy model, it will also be important to investigate if chronic administration of LOX and COX inhibitor is necessary to decrease seizure recurrence or if acute administration after SE is sufficient.

4.4 Translational Science Perspectives

As mentioned above, this study was designed in such a way that rats were dosed with inhibitors beginning two days before the SE induction; it was designed specifically for mechanistic work and has limitations just as any model does. Knowing this, it might be useful to highlight two hurdles this work will face if translation to clinical use is considered: clinical study operations and dose determination.

Since it is uncommon to use zileuton and celecoxib chronically, it is not foreseeable that a person would have two 4 doses of zileuton and celecoxib prior to a status epilepticus event. It would be clinically useful to know if beginning LOX and COX inhibition after seizure onset retains the ability to prevent barrier leakage. Therefore, as mentioned above, it will be important to establish a "treatment window" with numerous endpoint measures before considering a clinical study. If the effective window after SE is not long enough for patients to be easily enrolled, the study will struggle to meet adequate recruitment and it is essential to be able to demonstrate expected recruitment for success in obtaining funding.[96]

A benchtop challenge to the translational utility of these data is the impact of interspecies variation on dosing. Zileuton and celecoxib are both highly protein bound and extensively metabolized by the liver.[97] This complicates allometric dose scaling and additional pharmacokinetic modeling would be required to determine an appropriate dose for human study.[98] However, celecoxib and zileuton are both FDA-approved and therefore, some inferences can be made based on our dosing scheme and pharmacokinetic data already published. For example, rats given a single oral dose of 10 mg/kg celecoxib results in an $AUC_{0-\infty}$ of 18.5 µg*h/ml and a single 400 mg oral dose in humans results in an $AUC_{0-\infty}$ of 13.3 µg*h/ml.[97] The pharmacology data in the FDA approval package also suggest incomplete absorption of celecoxib when given orally. This means parenteral administration, as conducted in this study, would result in even higher exposures compared to those seen above. Although single doses were studied up to 1200 mg in humans, safety of multiple high doses has not been established and the highest label-approved regimen is 400 mg orally for one dose followed by 200 mg twice per day.[97] It will be prudent for clinical researchers and institutional review boards to consider the risks vs. benefits of an acute course of higher dosed celecoxib and zileuton, in a neurologic crisis such as SE, if dose scaling suggests FDA labeled dosing would not provide efficacy.

5. Conclusion

Current clinical and pre-clinical dogma states that seizures beget seizures and growing evidence support blood-brain barrier leakage as a contributing factor in this positive feedback loop. The data presented in this thesis provide compelling support for 5-LOX- and COX-2-controlled blood-brain barrier leakage in an *in vivo* SE model. The utility of zileuton and celecoxib for prevention of blood-brain barrier leakage is significant because these medications are already FDA-approved. This field would benefit from additional study to investigating 5-LOX and COX-2 as therapeutic drug targets to prevent blood-brain barrier leakage in epilepsy.

References

1. Zlokovic BV, Apuzzo ML. Strategies to circumvent vascular barriers of the central nervous system. Neurosurgery 1998;43:877-8.
2. Zack MM, Kobau R. National and State Estimates of the Numbers of Adults and Children with Active Epilepsy - United States, 2015. MMWR Morbidity and mortality weekly report 2017;66:821-5.
3. England MJ, Liverman CT, Schultz AM, Strawbridge LM. Summary: a reprint from epilepsy across the spectrum: promoting health and understanding. Epilepsy Curr 2012;12:245-53.
4. Hirtz D, Thurman DJ, Gwinn-Hardy K, Mohamed M, Chaudhuri AR, Zalutsky R. How common are the "common" neurologic disorders? Neurology 2007;68:326-37.
5. Kwan P, Brodie MJ. Early Identification of Refractory Epilepsy. New England Journal of Medicine 2000;342:314-9.
6. Fisher RS, Acevedo C, Arzimanoglou A, et al. ILAE official report: a practical clinical definition of epilepsy. Epilepsia 2014;55:475-82.
7. Selassie AW, Wilson DA, Martz GU, Smith GG, Wagner JL, Wannamaker BB. Epilepsy beyond seizure: a population-based study of comorbidities. Epilepsy research 2014;108:305-15.
8. Christensen J, Vestergaard M, Mortensen PB, Sidenius P, Agerbo E. Epilepsy and risk of suicide: a population-based case-control study. The Lancet Neurology 2007;6:693-8.
9. Hermann B, Seidenberg M, Jones J. The neurobehavioural comorbidities of epilepsy: can a natural history be developed? The Lancet Neurology 2008;7:151-60.
10. Jones JE, Hermann BP, Barry JJ, Gilliam FG, Kanner AM, Meador KJ. Rates and risk factors for suicide, suicidal ideation, and suicide attempts in chronic epilepsy. Epilepsy & Behavior 2003;4:31-8.
11. Trinka E, Cock H, Hesdorffer D, et al. A definition and classification of status epilepticus--Report of the ILAE Task Force on Classification of Status Epilepticus. Epilepsia 2015;56:1515-23.
12. Brophy GM, Bell R, Claassen J, et al. Guidelines for the evaluation and management of status epilepticus. Neurocritical care 2012;17:3-23.
13. Glauser T, Shinnar S, Gloss D, et al. Evidence-Based Guideline: Treatment of Convulsive Status Epilepticus in Children and Adults: Report of the Guideline Committee of the American Epilepsy Society. Epilepsy Currents 2016;16:48-61.
14. Treiman DM, Meyers PD, Walton NY, et al. A comparison of four treatments for generalized convulsive status epilepticus. Veterans Affairs Status Epilepticus Cooperative Study Group. N Engl J Med 1998;339:792-8.
15. Alldredge BK, Gelb AM, Isaacs SM, et al. A comparison of lorazepam, diazepam, and placebo for the treatment of out-of-hospital status epilepticus. N Engl J Med 2001;345:631-7.
16. Silbergleit R, Durkalski V, Lowenstein D, et al. Intramuscular versus intravenous therapy for prehospital status epilepticus. N Engl J Med 2012;366:591-600.
17. Cock HR. Established status epilepticus treatment trial (ESETT). Epilepsia 2011;52 Suppl 8:50-2.
18. Bleck T, Cock H, Chamberlain J, et al. The established status epilepticus trial 2013. Epilepsia 2013;54 Suppl 6:89-92.
19. Cook AM, Castle A, Green A, et al. Practice variations in the management of status epilepticus. Neurocritical care 2012;17:24-30.

20. Ferlisi M, Hocker S, Grade M, Trinka E, Shorvon S. Preliminary results of the global audit of treatment of refractory status epilepticus. Epilepsy Behav 2015;49:318-24.
21. DeLorenzo RJ, Pellock JM, Towne AR, Boggs JG. Epidemiology of status epilepticus. Journal of clinical neurophysiology : official publication of the American Electroencephalographic Society 1995;12:316-25.
22. Lv RJ, Wang Q, Cui T, Zhu F, Shao XQ. Status epilepticus-related etiology, incidence and mortality: A meta-analysis. Epilepsy research 2017;136:12-7.
23. Jafarpour S, Hodgeman RM, De Marchi Capeletto C, et al. New-Onset Status Epilepticus in Pediatric Patients: Causes, Characteristics, and Outcomes. Pediatric neurology 2017.
24. Raspall-Chaure M, Chin RF, Neville BG, Scott RC. Outcome of paediatric convulsive status epilepticus: a systematic review. The Lancet Neurology 2006;5:769-79.
25. Vilella L, Gonzalez Cuevas M, Quintana Luque M, et al. Prognosis of status epilepticus in elderly patients. Acta neurologica Scandinavica 2018;137:321-8.
26. Wu YW, Shek DW, Garcia PA, Zhao S, Johnston SC. Incidence and mortality of generalized convulsive status epilepticus in California. Neurology 2002;58:1070-6.
27. Gowers WR. Epilepsy and other Chronic Convulsive Diseases: Thier Causes, Symptoms, & Treatment. London: J. & A. Churchill; 1881.
28. Lawn N, Chan J, Lee J, Dunne J. Is the first seizure epilepsy--and when? Epilepsia 2015;56:1425-31.
29. Krumholz A, Wiebe S, Gronseth GS, et al. Evidence-based guideline: Management of an unprovoked first seizure in adults: Report of the Guideline Development Subcommittee of the American Academy of Neurology and the American Epilepsy Society. Neurology 2015;84:1705-13.
30. Chen Z, Brodie MJ, Liew D, Kwan P. Treatment Outcomes in Patients with Newly Diagnosed Epilepsy Treated With Established and New Antiepileptic Drugs: A 30-Year Longitudinal Cohort Study. JAMA neurology 2017.
31. Hesdorffer DC, Logroscino G, Cascino G, Annegers JF, Hauser WA. Risk of unprovoked seizure after acute symptomatic seizure: effect of status epilepticus. Ann Neurol 1998;44:908-12.
32. Verity CM, Ross EM, Golding J. Outcome of childhood status epilepticus and lengthy febrile convulsions: findings of national cohort study. BMJ (Clinical research ed) 1993;307:225-8.
33. Cavazzuti GB, Ferrari P, Lalla M. Follow-up study of 482 cases with convulsive disorders in the first year of life. Developmental medicine and child neurology 1984;26:425-37.
34. Lewandowski M. Zur Lehre der Cerebrospinalflussigeit. Z Klin Med 1990;40:480-94.
35. Ehrlich P. Sauerstoff-Bedürfniss des Organismus Eine farbenanalytische Studie. Berlin: August Hirschwald 1885.
36. Goldmann EE. Beitrag zur Physio-Pathologie des Plexus Chorioideus und der Hirnhäute. Abh Preuss Akad Wiss Physik 1913;1:1-60.
37. Reese TS, Karnovsky MJ. Fine structural localization of a blood-brain barrier to exogenous peroxidase. The Journal of cell biology 1967;34:207-17.
38. Brightman MW, Reese TS. Junctions between intimately apposed cell membranes in the vertebrate brain. The Journal of cell biology 1969;40:648-77.
39. Zhao Z, Nelson AR, Betsholtz C, Zlokovic BV. Establishment and Dysfunction of the Blood-Brain Barrier. Cell 2015;163:1064-78.

40. Tietz S, Engelhardt B. Brain barriers: Crosstalk between complex tight junctions and adherens junctions. The Journal of cell biology 2015;209:493-506.
41. Merali Z, Huang K, Mikulis D, Silver F, Kassner A. Evolution of blood-brain-barrier permeability after acute ischemic stroke. PloS one 2017;12:e0171558.
42. Jiang X, Andjelkovic AV, Zhu L, et al. Blood-brain barrier dysfunction and recovery after ischemic stroke. Progress in neurobiology 2017.
43. Sweeney MD, Sagare AP, Zlokovic BV. Blood-brain barrier breakdown in Alzheimer disease and other neurodegenerative disorders. Nat Rev Neurol 2018;14:133-50.
44. Marchi N, Teng Q, Ghosh C, et al. Blood-brain barrier damage, but not parenchymal white blood cells, is a hallmark of seizure activity. Brain Res 2010;1353:176-86.
45. Tomkins O, Shelef I, Kaizerman I, et al. Blood-brain barrier disruption in post-traumatic epilepsy. J Neurol Neurosurg Psychiatry 2008;79:774-7.
46. Rigau V, Morin M, Rousset MC, et al. Angiogenesis is associated with blood-brain barrier permeability in temporal lobe epilepsy. Brain : a journal of neurology 2007;130:1942-56.
47. van Vliet EA, da Costa Araujo S, Redeker S, van Schaik R, Aronica E, Gorter JA. Blood-brain barrier leakage may lead to progression of temporal lobe epilepsy. Brain : a journal of neurology 2007;130:521-34.
48. Marchi N, Angelov L, Masaryk T, et al. Seizure-promoting effect of blood-brain barrier disruption. Epilepsia 2007;48:732-42.
49. Angelov L, Doolittle ND, Kraemer DF, et al. Blood-brain barrier disruption and intra-arterial methotrexate-based therapy for newly diagnosed primary CNS lymphoma: a multi-institutional experience. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 2009;27:3503-9.
50. Kiechle K, Bazarian JJ, Merchant-Borna K, et al. Subject-specific increases in serum S-100B distinguish sports-related concussion from sports-related exertion. PloS one 2014;9:e84977.
51. Bouvier D, Duret T, Abbot M, et al. Utility of S100B Serum Level for the Determination of Concussion in Male Rugby Players. Sports medicine (Auckland, NZ) 2017;47:781-9.
52. Meier TB, Nelson LD, Huber DL, Bazarian JJ, Hayes RL, McCrea MA. Prospective Assessment of Acute Blood Markers of Brain Injury in Sport-Related Concussion. Journal of neurotrauma 2017;34:3134-42.
53. Petzold A, Green AJ, Keir G, et al. Role of serum S100B as an early predictor of high intracranial pressure and mortality in brain injury: a pilot study. Crit Care Med 2002;30:2705-10.
54. Korfias S, Stranjalis G, Boviatsis E, et al. Serum S-100B protein monitoring in patients with severe traumatic brain injury. Intensive care medicine 2007;33:255-60.
55. Carney N, Totten AM, O'Reilly C, et al. Guidelines for the Management of Severe Traumatic Brain Injury, Fourth Edition. Neurosurgery 2017;80:6-15.
56. Seiffert E, Dreier JP, Ivens S, et al. Lasting blood-brain barrier disruption induces epileptic focus in the rat somatosensory cortex. J Neurosci 2004;24:7829-36.
57. Ivens S, Kaufer D, Flores LP, et al. TGF-beta receptor-mediated albumin uptake into astrocytes is involved in neocortical epileptogenesis. Brain : a journal of neurology 2007;130:535-47.
58. van Vliet EA, Otte WM, Gorter JA, Dijkhuizen RM, Wadman WJ. Longitudinal assessment of blood-brain barrier leakage during epileptogenesis in rats. A quantitative MRI study. Neurobiology of disease 2014;63:74-84.

59. During MJ, Spencer DD. Extracellular hippocampal glutamate and spontaneous seizure in the conscious human brain. Lancet (London, England) 1993;341:1607-10.
60. Carlson H, Ronne-Engstrom E, Ungerstedt U, Hillered L. Seizure related elevations of extracellular amino acids in human focal epilepsy. Neuroscience letters 1992;140:30-2.
61. Hartz AM, Pekcec A, Soldner EL, Zhong Y, Schlichtiger J, Bauer B. P-gp Protein Expression and Transport Activity in Rodent Seizure Models and Human Epilepsy. Molecular pharmaceutics 2017;14:999-1011.
62. Bauer B, Hartz AM, Pekcec A, Toellner K, Miller DS, Potschka H. Seizure-induced up-regulation of P-glycoprotein at the blood-brain barrier through glutamate and cyclooxygenase-2 signaling. Molecular pharmacology 2008;73:1444-53.
63. !!! INVALID CITATION !!! {}.
64. Rempe RG, Hartz AMS, Soldner ELB, et al. Matrix Metalloproteinase-Mediated Blood-Brain Barrier Dysfunction in Epilepsy. J Neurosci 2018.
65. Curia G, Longo D, Biagini G, Jones RSG, Avoli M. The pilocarpine model of temporal lobe epilepsy. Journal of Neuroscience Methods 2008;172:143-57.
66. Jope RS, Morrisett RA, Snead OC. Characterization of lithium potentiation of pilocarpine-induced status epilepticus in rats. Experimental neurology 1986;91:471-80.
67. Honchar MP, Olney JW, Sherman WR. Systemic cholinergic agents induce seizures and brain damage in lithium-treated rats. Science (New York, NY) 1983;220:323-5.
68. Racine RJ. Modification of seizure activity by electrical stimulation. II. Motor seizure. Electroencephalography and clinical neurophysiology 1972;32:281-94.
69. Koeberle A, Siemoneit U, Buhring U, et al. Licofelone suppresses prostaglandin E2 formation by interference with the inducible microsomal prostaglandin E2 synthase-1. J Pharmacol Exp Ther 2008;326:975-82.
70. Eslami SM, Moradi MM, Ghasemi M, Dehpour AR. Anticonvulsive Effects of Licofelone on Status Epilepticus Induced by Lithium-pilocarpine in Wistar Rats: a Role for Inducible Nitric Oxide Synthase. Journal of epilepsy research 2016;6:51-8.
71. Hartz AM, Bauer B, Fricker G, Miller DS. Rapid regulation of P-glycoprotein at the blood-brain barrier by endothelin-1. Molecular pharmacology 2004;66:387-94.
72. Hartz AM, Bauer B, Soldner EL, et al. Amyloid-beta contributes to blood-brain barrier leakage in transgenic human amyloid precursor protein mice and in humans with cerebral amyloid angiopathy. Stroke 2012;43:514-23.
73. Marchi N, Rasmussen P, Kapural M, et al. Peripheral markers of brain damage and blood-brain barrier dysfunction. Restor Neurol Neurosci 2003;21:109-21.
74. Joseph SA, Lynd-Balta E, O'Banion MK, et al. Enhanced cyclooxygenase-2 expression in olfactory-limbic forebrain following kainate-induced seizures. Neuroscience 2006;140:1051-65.
75. Jiang J, Yang MS, Quan Y, Gueorguieva P, Ganesh T, Dingledine R. Therapeutic window for cyclooxygenase-2 related anti-inflammatory therapy after status epilepticus. Neurobiology of disease 2015;76:126-36.
76. Radu BM, Epureanu FB, Radu M, Fabene PF, Bertini G. Nonsteroidal anti-inflammatory drugs in clinical and experimental epilepsy. Epilepsy research 2017;131:15-27.
77. Marchi N, Betto G, Fazio V, et al. Blood-brain barrier damage and brain penetration of antiepileptic drugs: role of serum proteins and brain edema. Epilepsia 2009;50:664-77.

78. Salar S, Maslarova A, Lippmann K, et al. Blood-brain barrier dysfunction can contribute to pharmacoresistance of seizures. Epilepsia 2014;55:1255-63.
79. Friedman A, Heinemann U. Role of Blood-Brain Barrier Dysfunction in Epileptogenesis. In: Noebels JL, Avoli M, Rogawski MA, Olsen RW, Delgado-Escueta AV, eds. Jasper's Basic Mechanisms of the Epilepsies. 4th ed. Bethesda (MD)2012.
80. Gorter JA, van Vliet EA, Aronica E. Status epilepticus, blood-brain barrier disruption, inflammation, and epileptogenesis. Epilepsy Behav 2015;49:13-6.
81. Cacheaux LP, Ivens S, David Y, et al. Transcriptome profiling reveals TGF-beta signaling involvement in epileptogenesis. J Neurosci 2009;29:8927-35.
82. David Y, Cacheaux LP, Ivens S, et al. Astrocytic dysfunction in epileptogenesis: consequence of altered potassium and glutamate homeostasis? J Neurosci 2009;29:10588-99.
83. Raabe A, Schmitz AK, Pernhorst K, et al. Cliniconeuropathologic correlations show astroglial albumin storage as a common factor in epileptogenic vascular lesions. Epilepsia 2012;53:539-48.
84. Hinterkeuser S, Schroder W, Hager G, et al. Astrocytes in the hippocampus of patients with temporal lobe epilepsy display changes in potassium conductances. The European journal of neuroscience 2000;12:2087-96.
85. Cole AJ. Status epilepticus and periictal imaging. Epilepsia 2004;45 Suppl 4:72-7.
86. Bargerstock E, Puvenna V, Iffland P, et al. Is peripheral immunity regulated by blood-brain barrier permeability changes? PloS one 2014;9:e101477.
87. Fabene PF, Navarro Mora G, Martinello M, et al. A role for leukocyte-endothelial adhesion mechanisms in epilepsy. Nature medicine 2008;14:1377-83.
88. Marchi N, Tierney W, Alexopoulos AV, Puvenna V, Granata T, Janigro D. The etiological role of blood-brain barrier dysfunction in seizure disorders. Cardiovasc Psychiatry Neurol 2011;2011:482415.
89. Fabene PF, Bramanti P, Constantin G. The emerging role for chemokines in epilepsy. J Neuroimmunol 2010;224:22-7.
90. Zattoni M, Mura ML, Deprez F, et al. Brain infiltration of leukocytes contributes to the pathophysiology of temporal lobe epilepsy. J Neurosci 2011;31:4037-50.
91. Shih AY, Driscoll JD, Drew PJ, Nishimura N, Schaffer CB, Kleinfeld D. Two-photon microscopy as a tool to study blood flow and neurovascular coupling in the rodent brain. Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism 2012;32:1277-309.
92. Smith QR, Allen DD. In situ brain perfusion technique. Methods in molecular medicine 2003;89:209-18.
93. Koeberle A, Laufer SA, Werz O. Design and Development of Microsomal Prostaglandin E2 Synthase-1 Inhibitors: Challenges and Future Directions. Journal of medicinal chemistry 2016;59:5970-86.
94. Jin Y, Smith CL, Hu L, et al. Pharmacodynamic comparison of LY3023703, a novel microsomal prostaglandin e synthase 1 inhibitor, with celecoxib. Clinical pharmacology and therapeutics 2016;99:274-84.
95. Yokomizo T, Izumi T, Shimizu T. Leukotriene B4: metabolism and signal transduction. Archives of biochemistry and biophysics 2001;385:231-41.
96. Cofield SS, Conwit R, Barsan W, Quinn J. Recruitment and retention of patients into emergency medicine clinical trials. Academic emergency medicine : official journal of the Society for Academic Emergency Medicine 2010;17:1104-12.
97. Celebrex Approval Package. United States Food and Drug Administration 2001;21-156.

98. Sharma V, McNeill JH. To scale or not to scale: the principles of dose extrapolation. British journal of pharmacology 2009;157:907-21.

Vita

EDUCATION

Bachelor of Science in Cell and Molecular Biology — 2009 – 2013
University of Minnesota – Duluth — GPA – 3.41
Duluth, MN

AWARDS

Dean's List — 2017
University of Kentucky College of Pharmacy

Kappa Psi Scholarship Honors Certificate — 2017

Summer Research Program — 2016
University of Kentucky College of Pharmacy

Kappa Psi Scholarship Honors Certificate — 2016

Dean's List — 2014
University of Kentucky College of Pharmacy

PUBLICATIONS

1. Rempe RG, Hartz AMS, Soldner ELB, **Sokola BS**, Alluri SR, Abner EL, Kryscio RJ, Pekcec A, Schlichtiger J, Bauer B. Matrix Metalloproteinase-Mediated Blood-Brain Barrier Dysfunction in Epilepsy. *J Neurosci.* epub ahead of print. 2018

2. Ma DW, Wang AC, Parikh I, Green SJ, Hoffman JD, Chipala G, **Sokola BS**, Bauer B, Hartz AMS, Lin AL. Early-Interventional Ketogenic Diet Enhances Brain Function and Alters Gut Microbiome in Healthy Mice. *Sci. Rep.* epub ahead of print. 2018.

3. Hartz AMS, Schulz JA, **Sokola BS**, Edelmann S, Shen AN, El Seblani N, Rempe RG, Zhong Y, Bauer B. Isolation of Human Brain Capillaries.
   - Accepted – *J Vis Exp.*

4. **Sokola BS**, Rempe RG, Hartz AMS, Carney K, Bauer B. LOX/COX Inhibition Restores Blood-Brain Barrier Function in Epilepsy.
   - Manuscript in preparation

5. Hartz AMS, Schulz JA, **Sokola BS**, Samala R, Bauer B. Blood-Brain Barrier Transporter P-gp and BCRP are Regulated by PI3K/Akt Signaling
   - Manuscript in preparation