

WSU Program for TBI Research Summer School

Neuroprotection & Mechanism of Ethanol Therapy in Stroke/TBI: New Prospects for an Ancient Drug

Yuchuan Ding, MD, PhD

Department of Neurological Surgery

June 7, 2013




History of Alcohol (Ethanol)

- The earliest evidence of alcohol (ethanol) use is the discovery of beer jugs from the Neolithic age, dated to approximately 10,000 B.C.
- Various evidence suggests that ethanol was introduced in the Near East. The people of Mesopotamia obtained the necessary fermentable sugar from wild berries, grapes, and tree sap, which were readily available in the warm climates of the Near East.
- The use of alcohol spread to Persia, where the first wine was made, sometime between 5,400 and 5,000 B.C.
- Through trade and travel, alcohol was then carried to ancient Egypt around 4,000 B.C. The Ancient Egyptians used alcohol on a daily basis, believing that wine was a creation of the god Osiris. *They were the first to recognize its therapeutic benefits as a painkiller and digestive aid.*
- By 2,500 B.C., Sumerians and Babylonians used alcohol as an offering to their gods, as evidenced in the code of Hammurabi.

History of Alcohol (Ethanol)

- Alcoholic beverages finally spread to Europe via Greece by 1,700 B.C.
- The founder of Western medicine, Hippocrates, used low to moderate doses of alcohol to treat various acute and chronic conditions including pneumonia, jaundice, anemia, and menstrual pains.*
- Alcohol was also recommended as an effective antiseptic for wounds as early as 200 AD by *Claudius Galen*. This use persisted throughout the centuries, though it was not medically explored until the late 1800s and not verified until the late 1900s.
- In modern medicine, ethanol has been used clinically as an antidote to toxic alcohol (e.g., methanol) ingestion.*

Why Alcohol (Ethanol)?

Epidemiologic studies have proposed a role for ETOH as both a risk factor and a potential protective factor for TBI/stroke

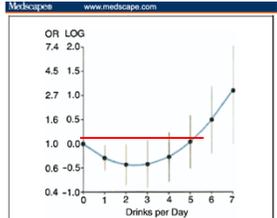


Figure 3 Alcohol and Stroke Risk

Relationship between daily alcohol and ischemic stroke. This was fully adjusted for the usual stroke factors. OR = odds ratio. Reproduced with permission from Sacco et al. (12).

Source: JACC © 2007 American College of Cardiology Foundation

Source: http://www.medscape.com/viewarticle/562474_2

J-shaped Relationship Between Alcohol Consumption and Stroke

American Journal of medicine 1991: Gill et al

- Low and Moderate alcohol levels - Decreased relative risk of stroke
- Heavy consumption- Elevated risk of Stroke
- Pattern same for sub-arachnoid hemorrhage, intracerebral hemorrhage, and cerebral infarction
- Independent of major confounding factors

Why ethanol?

Serum Ethanol Levels: Predictor of Survival After Severe Traumatic Brain Injury

J Trauma. 2009 Oct;67(4):697-703.

Ali Salim, MD, Pedro Teixeira, MD, Eric J. Ley, MD, Joseph DuBose, MD, Kenji Inaba, MD, and Daniel R. Margulies, MD

Alcohol in Moderation, Cardioprotection, and Neuroprotection: Epidemiological Considerations and Mechanistic Studies

Michael A. Collins, Edward J. Neafsey, Kenneth J. Mukherjee, Mary O. Grisy, Dale A. Parko, Dipak K. Das, and Ronald J. Koob

Association Between Alcohol and Mortality in Patients With Severe Traumatic Head Injury

Arch Surg. 2006 Dec;141(12):1185-91

Homer C. N. Tien, MD, FRCSC; Lorraine N. Tremblay, MD, PhD; Sandro B. Rizoli, MD, PhD; Jacob Gelberg, BSc; Talat Chughtai, MD; Peter Tikuisis, PhD; Pang Shek, PhD; Frederick D. Bremner, MD

Why Alcohol (Ethanol)?

Correlating the Blood Alcohol Concentration with Outcome after Traumatic Brain Injury: Too Much Is Not a Bad Thing

CHERRISE BERRY, M.D., ERIC J. LEY, M.D., DANIEL R. MARGULIES, M.D., JAMES MROCHKA, MARKO BUKUR, M.D., DARREN MALINOSKI, M.D., ALI SALIM, M.D.

From the Department of Surgery, Division of Trauma and Critical Care, Cedars-Sinai Medical Center, Los Angeles, California

Although recent evidence suggests a beneficial effect of alcohol for patients with traumatic brain injury (TBI), the level of alcohol that confers the protective effect is unknown. Our objective was to investigate the relationship between admission blood alcohol concentration (BAC) and outcomes in patients with isolated moderate to severe TBI. From 2005 to 2009, the Los Angeles County Trauma Database was queried for all patients ≥ 14 years of age with isolated moderate to severe TBI and admission serum alcohol levels. Patients were then stratified into four levels based on admission BAC: None (0 mg/dL), low (0-100 mg/dL), moderate (100-230 mg/dL), and high (≥ 230 mg/dL). Demographics, patient characteristics, and outcomes were compared across levels. In evaluating 3288 patients, the mortality rate decreased with increasing BAC levels (linear trend $P < 0.0001$). In determining the relationship between BAC and mortality, multivariable logistic regression analysis demonstrated a high BAC level was significantly protective (adjusted odds ratio 0.55; 95% confidence interval: 0.38-0.8; $P = 0.002$). In the largest study to date, a high (≥ 230 mg/dL) admission BAC was independently associated with improved survival in patients with isolated moderate to severe TBI. Additional research is warranted to investigate the potential therapeutic implications.

The American Surgeon; 2011; 77 (10)

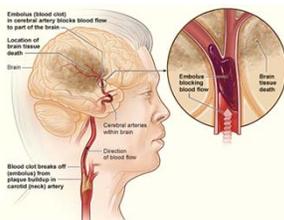
Why Alcohol (Ethanol)?

- Heavy alcohol consumption was linked to an increased risk of total stroke incidence, ischemic and hemorrhagic stroke.
- The association between light-to-moderate alcohol consumption and stroke, was conflicting: either inversely related to risk of total stroke, ischemic stroke and hemorrhagic stroke, or proportionally related to the risk of stroke.
- Antecedent ETOH treatment (an alcohol pre-conditioning) attenuated post-ischemic neuronal injury and behavioral deficit.
- In traumatic brain injury (TBI), a preconditioning-type neuroprotection was observed. Elevated serum ethanol levels were associated with increased survival in patients with moderate to severe brain injury, suggesting that *prior* exposure to a relatively high dose of alcohol is neuroprotective.

Hypothesis

- **One time dose of alcohol administered within a given therapeutic window after stroke or TBI result in better outcome.**

Ischemic Stroke and Therapeutic Targets



- No. 3 cause of death worldwide
- No. 1 cause of disability
- Ischemic stroke accounts for approximately 70-80% of all strokes (ischemic and hemorrhagic)

Cerebral infarction may ensue within minutes of a critical reduction in cerebral blood flow.

MCA reportedly is the most common site of obstruction in ischemic stroke.

Up to 78% of patients with acute stroke due to MCA occlusion die or become severely disabled.

Stroke data in the U.S.

- 795,000 strokes a year
 - 610,000 are first or new strokes
 - 185,000 are recurrent
- Fourth leading cause of death.
- There is 1 stroke every 40 seconds and 1 death due to stroke every 4 minutes.
- \$53.9 billion in related costs in 2010.

Stroke Treatments

• Ideally, "the treatment of acute ischemic stroke will require not only fixing the plumbing but also impeding the tissue and cellular consequences induced by the vascular occlusion and its removal" (M. Fisher 2012).

• Cerebrovascular thrombolysis with intravenous (IV) tissue plasminogen activator (tPA) and *in situ* clot retrieval are the only stroke treatments approved by FDA as **reperfusion strategies**. However, most patients (>90%) with acute ischemic stroke do not benefit from a reperfusion strategy because of treatment contraindications, the narrow therapeutic time window and the reperfusion injury.

• No **neuroprotective strategies** (drugs) so far has proven effective in phase III trials despite promise in animal studies.



Aims

- **Development of Neuroprotective Treatment in Acute Ischemic Stroke.**

A Effective Neuroprotective Therapy Should be:

- Easy to administer and cross BBB
- Safe
- Improves patient outcome significantly
- No interruption with current therapy, such as tPA

Alcohol Dosage

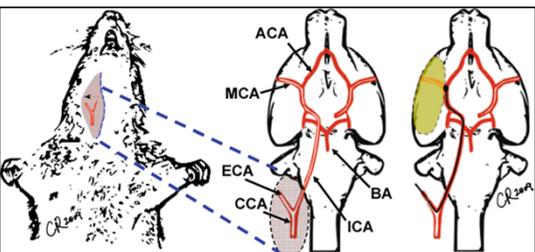
One Standard Drink Equals 12 Grams of Pure Alcohol		Drinks Equal Mass of the Body of Alcohol	
 Beer	8 oz.	1 Drink	0.30 g/kg
 Wine	4 oz.	2 Drinks	0.60 g/kg
 Hard Liquor (80 proof)	1 oz.	3 Drinks	0.90 g/kg
		4 Drinks	1.20 g/kg
		5 Drinks	1.50 g/kg

Alcohol Dosage and Blood Concentration

- 1.5 g/kg represents the threshold concentration above which a person is legally drunk when operating a motor vehicle (0.80mg/mL, 80mg/dL, 0.08%) in USA

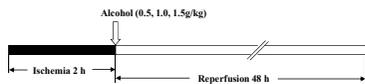
Mass of alcohol per mass of the body		Mass per volume of Blood in the body
0.5 g/kg	1-2 drinks	0.40 mg/mL 40 mg/dL
1.0 g/kg	3-4 drinks	0.56 mg/mL 56 mg/dL
1.5 g/kg	5 drinks	0.89 mg/mL 89 mg/dL

Stroke Model System

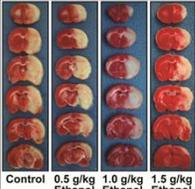
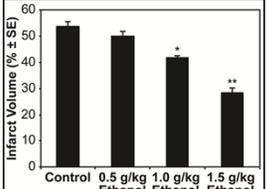


MCAO surgery on the Sprague Dawley rat

Determine The Optimal Therapeutic Dose Of Alcohol

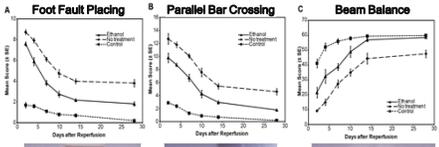


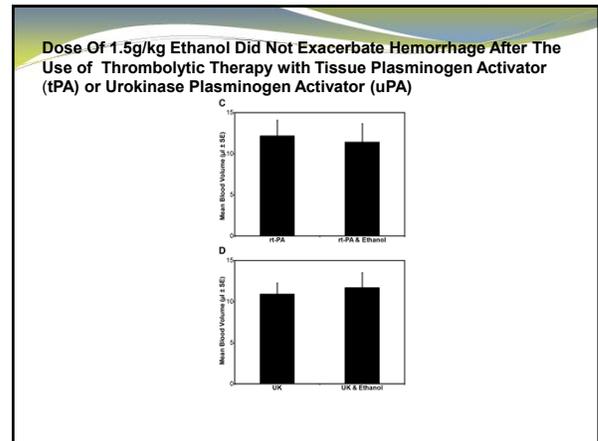
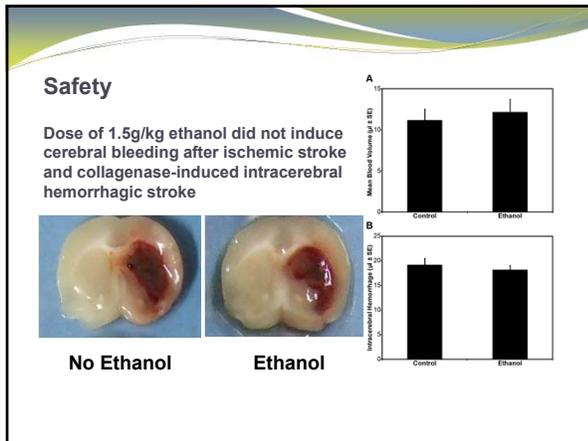
Infarct Volume

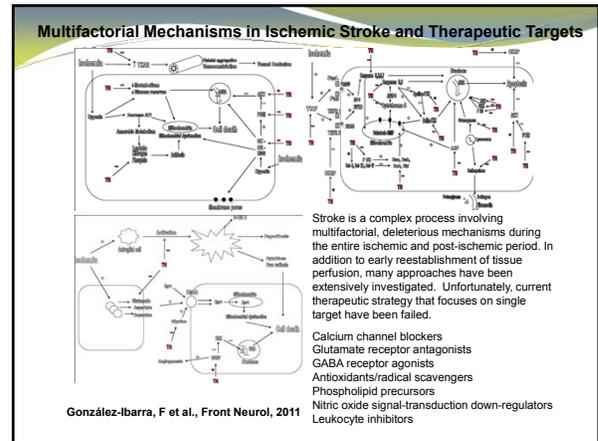
Neuroprotective effect of acute ethanol administration in a rat with transient cerebral ischemia. Stroke (2012) 43, 205-210.

Alcohol of 1.5g/kg Improve Motor Functions After Stroke



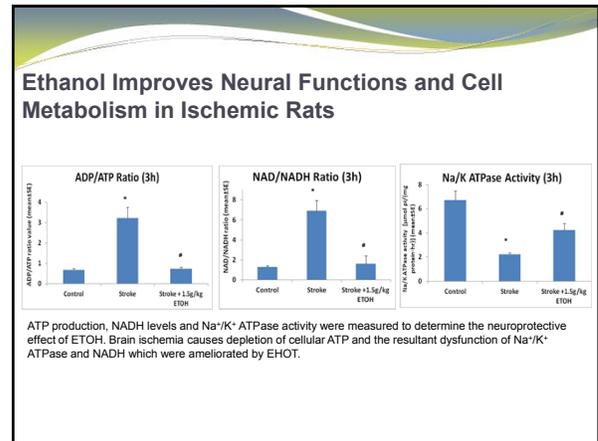
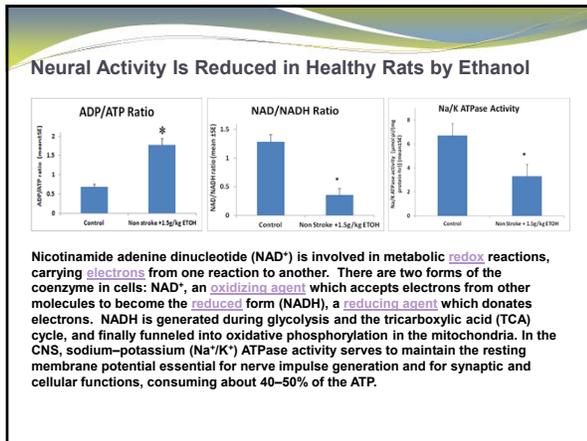
What is the Mechanism(s) underlying Ethanol-induced Neuroprotection?



- To understand the mechanisms how a single molecule, ETOH, affects multiple molecular pathways, leading to a strong neuroprotection.
- To study the feasibility for use of ETOH, the ancient drug, as a new neuro-protectant, which may lead to a development of a new and effective stroke therapy beyond the levels achieved by previous studies.

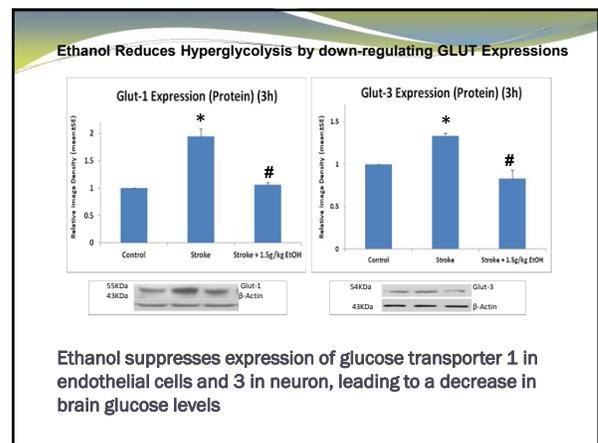
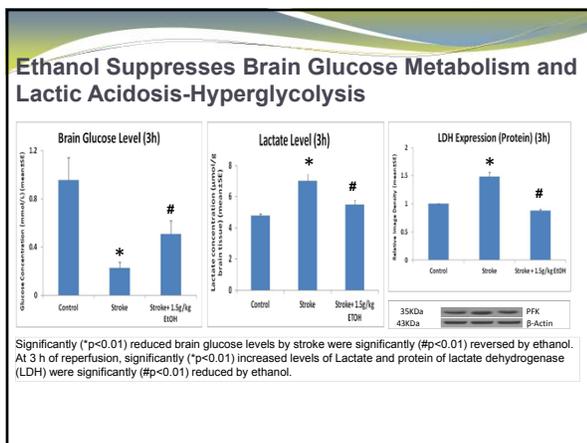
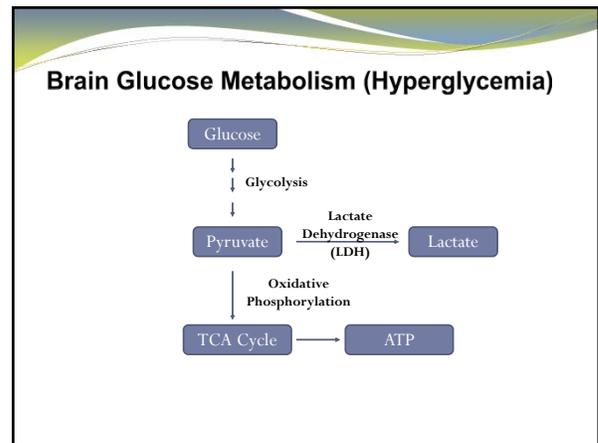
Hypothesis 1:

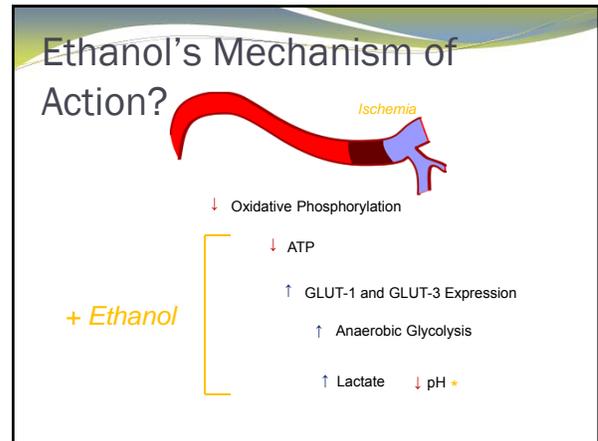
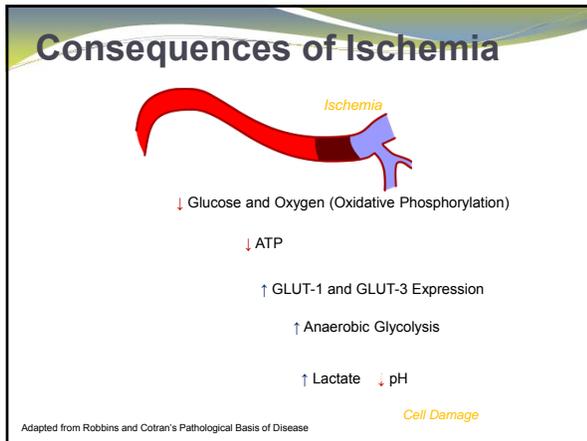
Ethanol Reduces Cerebral Metabolism; Reduce Energy Demands



Hypothesis 2:

Ethanol Reduces Hyperglycolysis, Ameliorating Lactic Acid-induced Cellular Acidosis



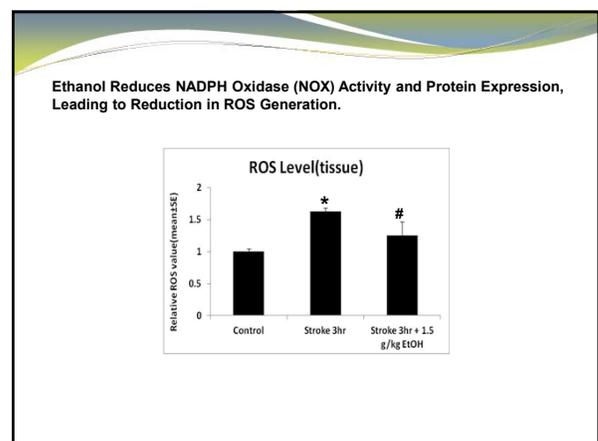
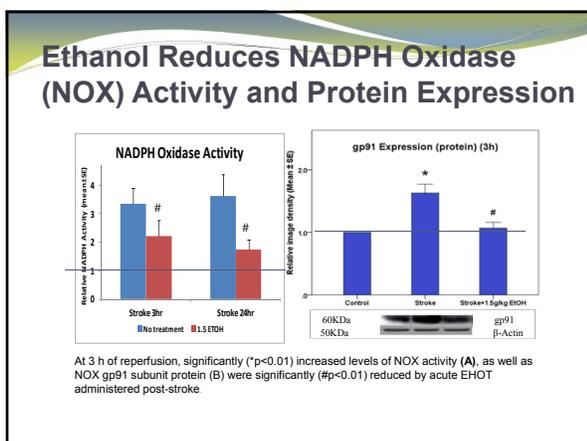


Hypothesis 3:

Ethanol Reduces NADPH Oxidase Activity and Protein Expression, Ameliorating Reactive Oxygen Species (ROS) generation

Nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (NOX) Activation Leads to ROS Generation

NOX, a membrane-bound enzyme complex located both in cytosol and plasma membrane, plays pivotal roles in cerebral ischemia/reperfusion injury by promoting ROS generation, which, in turn, causes oxidative stress and neuronal death.



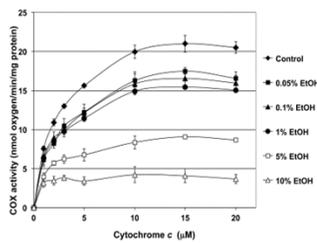
Hypothesis 4

Ethanol Reduces Hyperactive Activity of Mitochondrial Enzyme (cytochrome c oxidase) in Ischemia/Reperfusion Injury

Hypothesis 4

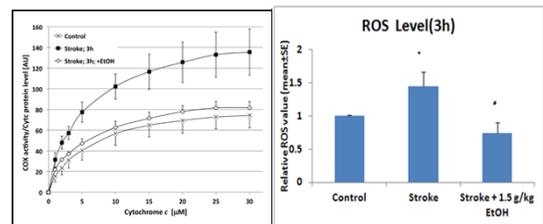
In mitochondria, the energy house, cytochrome c oxidase (CcO), the main regulator of oxidative phosphorylation, becomes hyperactive during ischemia, leading to the mitochondrial membrane potential hyperpolarization, which, in turn, especially immediately after reperfusion, generates excessive ROS.

Cytochrome c Oxidase Regulation by Ethanol



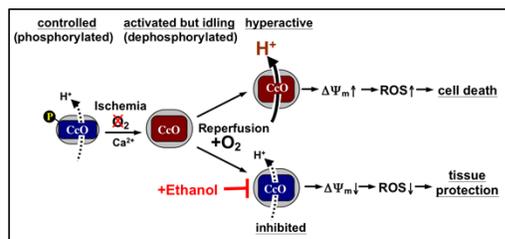
Ethanol leads to CcO inhibition with isolated cow liver/brain-type COX.

Cytochrome c Oxidase Regulation

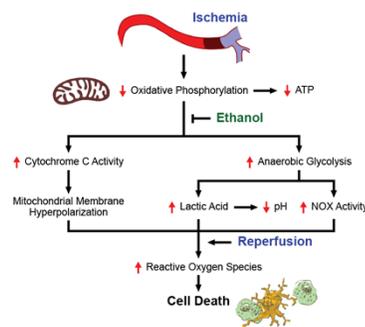


In ischemic rats, CcO activity was determined in brain homogenates and significantly ($p < 0.05$) increased after ischemia/reperfusion, as compared to non-ischemic control rats. The effect was significantly ($p < 0.05$) reversed by ethanol, in association with reduced ROS..

Ethanol's Mechanism of Action: CcO Regulation

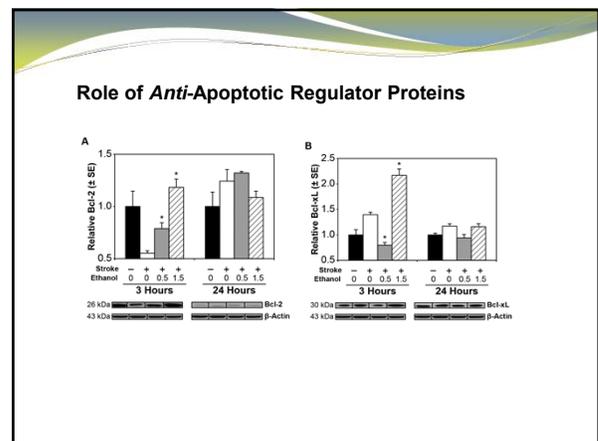
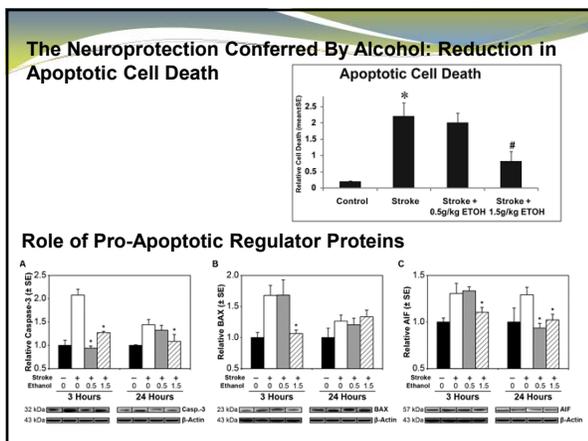
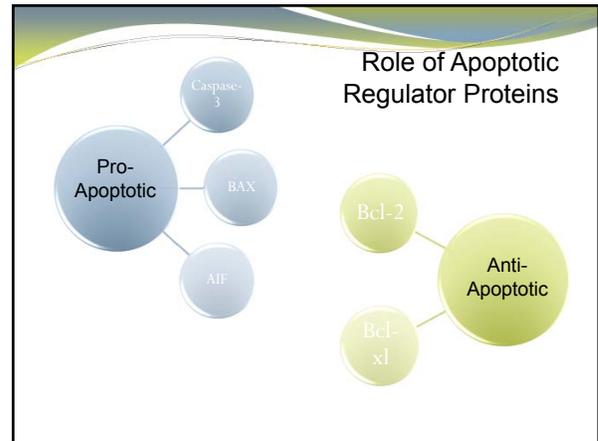
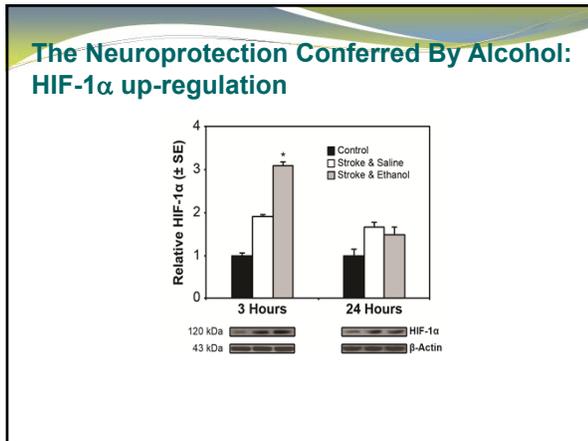
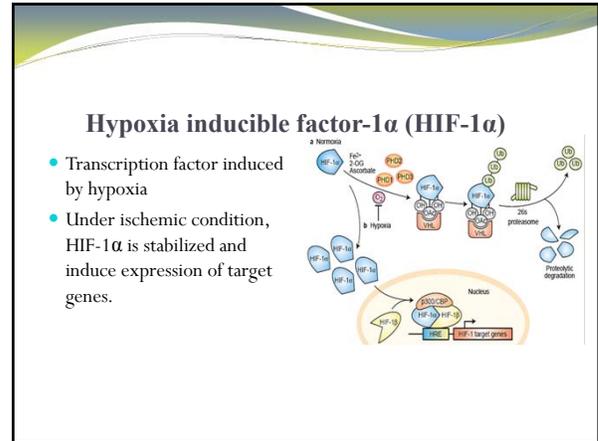


Summary



Hypothesis 5

Ethanol Up-regulates Hypoxia inducible factor-1 α (HIF-1 α) and Down-regulates Apoptotic Protein Expressions in Ischemia/reperfusion Injury



Summary

- Previous studies have shown:
 - J-shaped curve between Alcohol consumption and Stroke risk
 - High doses of alcohol are associated with increased risk of stroke
 - Mild to moderate doses of alcohol are neuroprotective
- Our study establishes the benefits of acute alcohol administration
 - Alcohol administration after stroke decreases brain injury
 - *Multiple* mechanisms may contribute to ethanol's beneficial effect, including improved brain energy balance, glucose metabolism and mitochondria activity, as well as increases in neuroprotective cytokines and decreases in pro-apoptotic proteins.

This studies enhance the prospect for the use of this ancient drug on its new, novel, therapeutic feasibility in stroke.

ETHANOL

↑
HIF-1α

↑
Hyperglycolysis

↓
Apoptosis

↑
BBB Dysfunction

↑
Brain Metabolism

Brain Edema After Traumatic Brain Injury

- **In severe TBI, brain edema poses a critical clinical problem due to its association with increased morbidity and mortality. Despite its clinical importance, all present treatment options for edema only provide symptomatic relief.**
- **Whether ethanol play a beneficial role in reducing brain edema formation and what is the underlying mechanisms.**

Traumatic Brain Injury (TBI): Marmarou's Rat Acceleration Impact Model

Reduced Brain Edema by Ethanol administration

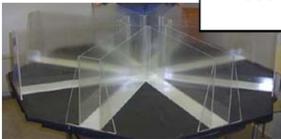
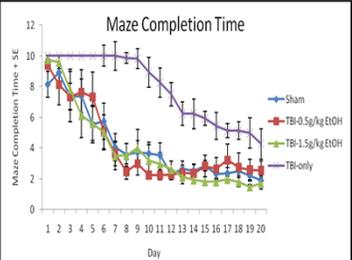
Edema

Group	Water Content Percentage (means±SE)
Control	~74.5
TBI	~78.5*
TBI+0.5 g/kg ETOH	~77.5#
TBI+1.5 g/kg ETOH	~77.0#

Determine Functional Outcome after TBI with Alcohol

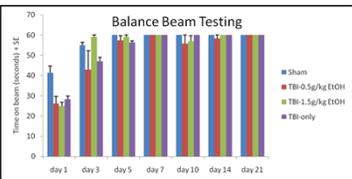
Cognitive Behavior in TBI w/ or w/o Treatments

The radial arm maze was used. TBI animals showed significantly ($p < 0.05$) poorer performance than sham-operated animals and treatment groups.

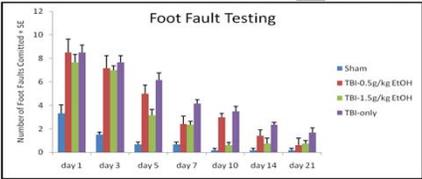
Day	Sham	TBI-0.5g/kg EtOH	TBI-1.5g/kg EtOH	TBI-only
1	10	10	10	10
2	8	9	9	9
3	6	8	8	8
4	5	7	7	7
5	4	6	6	6
6	3	5	5	5
7	3	4	4	4
8	3	4	4	4
9	3	4	4	4
10	3	4	4	4
11	3	4	4	4
12	3	4	4	4
13	3	4	4	4
14	3	4	4	4
15	3	4	4	4
16	3	4	4	4
17	3	4	4	4
18	3	4	4	4
19	3	4	4	4
20	3	4	4	4

Motor Balance in TBI Rats w/or w/o Treatments

Rats were placed on the wood beam and their time remaining on the beam up to 60 seconds was recorded. We found that Ethanol at 1.5g/kg but not 0.5g/kg dose significantly reduced the balancing deficits as early as 3 day after TBI.

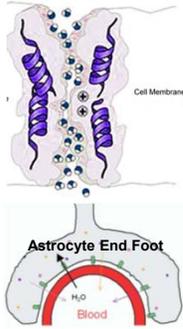
Foot Fault Testing

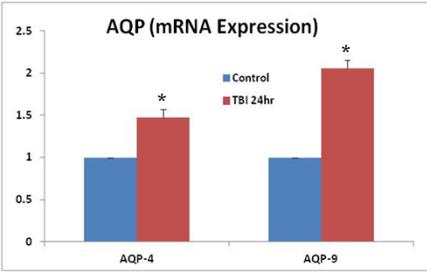
Foot Fault Test after TBI with or without Ethanol to examine sensorimotor integration motor balance, and co-ordination in forelimb and hindlimb placing in response to visual, tactile and proprioceptive stimuli. Deficits in this test assess coordination rather than activity. TBI causes coordination deficits; Ethanol (1.5g/kg) significantly reduced the deficits at day 5 and day 7 for both doses. Up to day 21, TBI rats still have significant deficits.

Aquaporin (AQP)

- Water channel proteins provide a key route for water movement (Peter Agre, M.D., professor of biological chemistry at the Johns Hopkins University School of Medicine, was awarded the 2003 Nobel Prize in Chemistry).
- AQP-4 and 9 are abundant in brain and expressed in astrocyte foot processes and nearby capillaries (endothelial cells).
- Inhibition of AQP4 and AQP9 reduces brain edema, secondary neuronal damage and functional deficits after TBI.

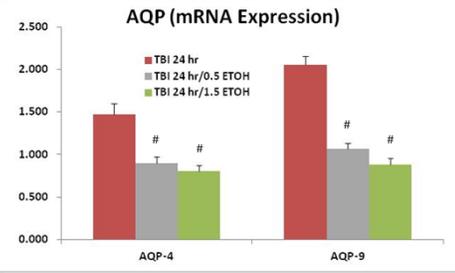


AQP mRNA Expression determined by Real-time PCR

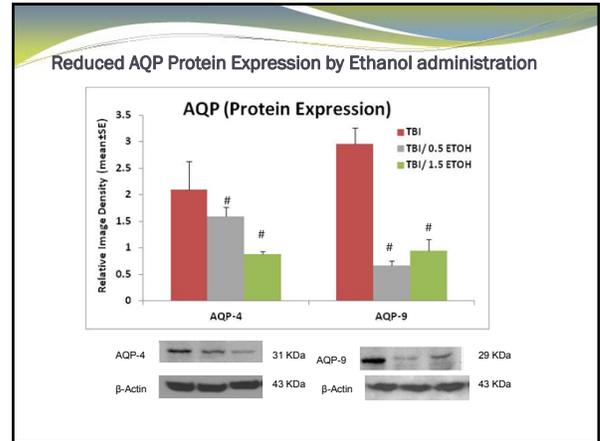
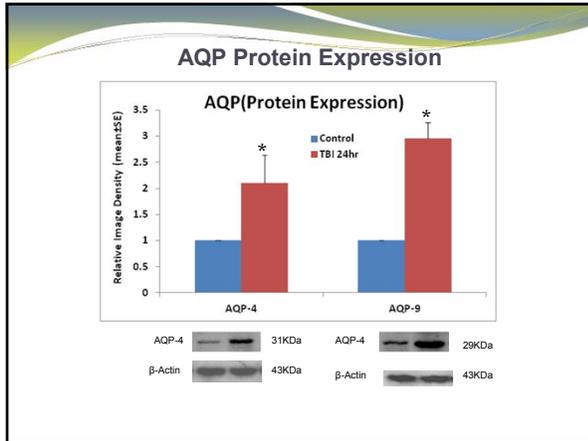


Group	AQP-4	AQP-9
Control	1.0	1.0
TBI 24hr	~1.5*	~2.1*

AQP mRNA Expression determined by Real-time PCR



Group	AQP-4	AQP-9
TBI 24 hr	~1.5	~2.1
TBI 24 hr/0.5 EtOH	~0.9#	~1.1#
TBI 24 hr/1.5 EtOH	~0.8#	~0.9#



Summary for TBI Study

- One time alcohol administration within 1 hour after TBI results in better outcome.
- Administration of alcohol reduce brain edema by inhibiting AQP-4 and -9 expression after TBI

Thank you

