Ethanol Administration after Stroke Regulates Mitochondrial Oxidative Phosphorylation by Targeting Cytochrome c Oxidase and Pyruvate Dehydrogenase

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Introduction

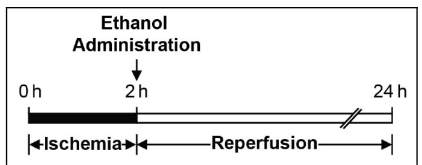
Ischemia/reperfusion injury following stroke onset produces a dysfunction in the metabolic state of the neuronal cells leading to mitochondrial impairment and hence reduced energy production. When administered at the onset of reperfusion, ethanol reduces brain infarct size and improves functional outcome. In this study, we examine the effect of ethanol on mitochondrial dysfunction following an ischemic stroke.

Figure 1. Ethanol decreases infarct volume in rat. **A**. Rat brain slice stained for visualization of infarct volume. 48 h after reperfusion. **B**. Quantitative analysis of infarct volume. Adapted from: Stroke. 2012 43(1):205-10.

Figure 1. Infarct Volume and Ethanol

Methods

An ischemic stroke model was generated by occlusion of the right middle cerebral artery for two hours in male Sprague-Dawley rats. Ethanol was administered immediately at the onset of reperfusion (Scheme 1). Membrane Na+/K+ ATPase activity, ADP:ATP ratio, and NAD:NADH ratio were measured to assess the cellular metabolic state... Expression of pyruvate dehydrogenase (PDH) was measured using Western blot analysis. Reactive oxygen species (ROS) formation was measured by fluorometric techniques. Cytochrome c oxidase activity was also assayed.



Scheme 1. Procedure Timeline

Results

1. Ethanol treatment produced a significant (P<0.05) decrease in the ADP:ATP and NAD:NADH ratios, increase in the membrane Na+/K+ ATPase activity, and increase in PDH expression when compared with saline-treated rats (Figures 2, 3, 4 and 5):

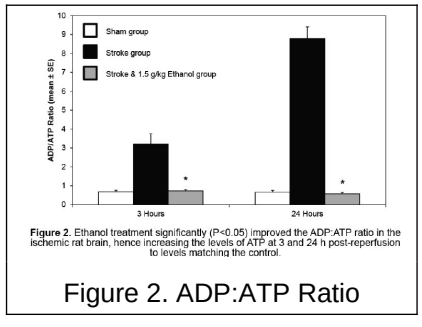


Figure 2. Ethanol treatment significantly (P<0.05) improved the ADP:ATP ratio in the ischemic rat brain, hence increasing the levels of ATP at 3 and 24 h post-reperfusion to levels matching the control.

Figure 2. ADP:ATP Ratio

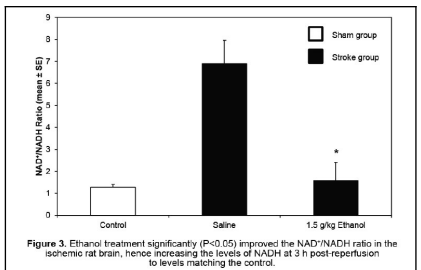


Figure 3. Ethanol treatment significantly (P<0.05) improved the NAD+/NADH ratio in the ischemic rat brain, hence increasing the levels of NADH at 3 h post-reperfusion to levels matching the control.

Figure 3. NAD/NADH Ratio

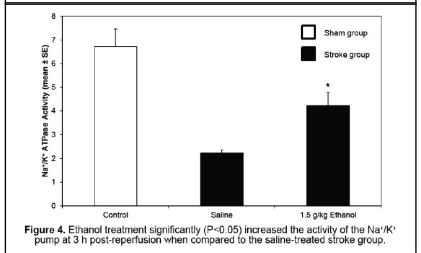


Figure 4. Ethanol treatment significantly (P<0.05) increased the activity of the Na+/K+ pump at 3 h post-reperfusion when compared to the saline-treated stroke group.

Figure 4. Na/K ATPase Activity

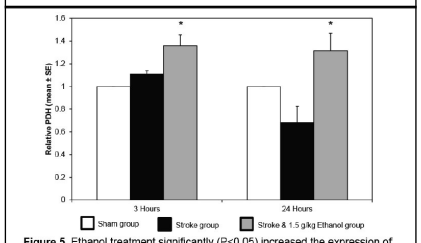


Figure 5. Ethanol treatment significantly (P<0.05) increased the expression of pyruvate dehydrogenase (PDH) at 3 h and at 24 h after the initiation of reperfusion.

Figure 5. PDH Expression

2. Ethanol treatment, when compared to saline treatment, showed significantly (P<0.05) lower rates of mitochondrial ROS production (Figure 6):

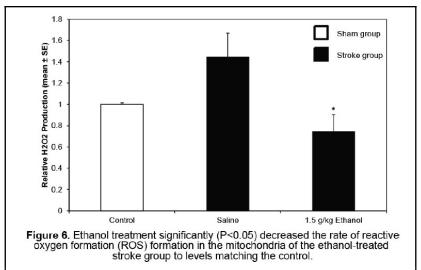


Figure 6. Ethanol treatment significantly (P<0.05) decreased the rate of reactive oxygen formation (ROS) formation in the mitochondria of the ethanol-treated stroke group to levels matching the control.

Figure 6. Mitochondrial ROS Production

3. The activity of cytochrome c oxidase, a key regulator of oxidative phosphorylation, was found to be significantly reduced to levels matching the control in the ethanol-treated stroke group (Figure 7):

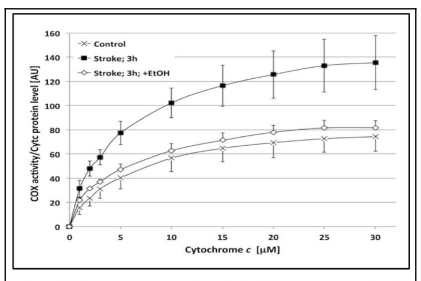
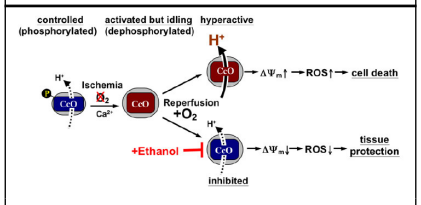


Figure 7. Ethanol treatment significantly (P<0.05) reduces the activity of Cytochrome c oxidase at 3 h after reperfusion to levels matching the control, preventing mitochondrial hyperpolarization.

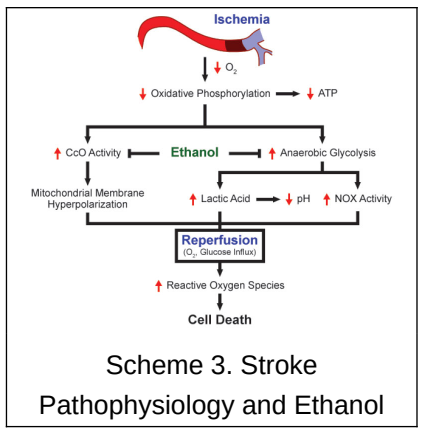
Figure 7. Cytochrome c Oxidase Activity



Scheme 2. Cytochrome c Oxidase Regulation

Conclusions

Ethanol at concentrations that are close to the legal driving limit exerts a strong neuroprotective effect by regulating and preserving mitochondrial oxidative phosphorylation to minimize ROS production and maximize efficiency, providing neurons with adequate levels of ATP.



Scheme 3. Stroke Pathophysiology and Ethanol

Learning Objectives

By the conclusion of this session, participants should be able to: 1) Describe the possibility of using ethanol in treating stroke, 2) Hypothesize the mechanism of protection offered by this agent, 3) Provide a future direction for acute ethanol treatment in stroke.

References

1. Wang F, Wang Y, Geng X, et al. Neuroprotective effect of acute ethanol administration in a rat with transient cerebral ischemia. Stroke 2012;43:205-210.