



1923

Effect of Endogenous Adenosine Augmentation on Ischemia and Reperfusion Injury to the Liver

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ADENOSINE is known to cause vasodilation and to inhibit neutrophil activation, platelet aggregation, endothelin production, and superoxide production in the myocardium after ischemia and reperfusion.¹ To exploit these biological effects of adenosine to prevent tissue injury from ischemia and reperfusion, exogenous adenosine supplementation and endogenous adenosine supplement have been used experimentally. The aim of this study is to determine whether endogenous adenosine supplementation by inhibition of nucleoside transport (R75231) or adenosine deaminase (EHNA) can attenuate ischemia/reperfusion injury to the canine liver.

MATERIALS AND METHODS

Two-hour complete hepatic ischemia was induced in adult female beagle dogs by clamping the vena cava (above and below the liver), and the portal triad. Splanchnic and inferior systemic venous beds were decompressed using veno-venous bypass. R75231 at a dose of 0.05 mg/kg ($n = 5$, Janssen Research Foundation, Beerse, Belgium) and EHNA at a dose of 10 mg/kg ($n = 5$, Sigma, St Louis, Mo) were given as a continuous intravenous infusion (via a peripheral vein) over 30 minutes, just before hepatic ischemia. Control animals ($n = 10$) received no treatment. Animal survival, hepatic tissue blood flow, liver function, adenine nucleotides, purine catabolites and histopathology were analyzed. Results are presented as the mean \pm the standard error of the mean. Statistical analysis of the results was performed using the Log Rank test (for survival), and the Mann-Whitney U test (all others). A p value less than .05 was considered significant.

RESULTS

Two-week animal survival in the Control group (30%) was significantly worse than the R75231 group (100%), but not different from the EHNA group (60%). Peak AST and ALT levels in the Control and EHNA groups were significantly higher than AST and ALT levels in the R75231 group (Fig 1a). During and after ischemia, adenosine levels and adenine nucleotide levels in the EHNA group were significantly higher than the levels in the Control and R75231 groups. However, hypoxanthine levels in the R75231 group were significantly lower than the Control and EHNA groups. The recovery of energy charge, and adenine nucleotides, particularly ATP, after reperfusion was significantly better in the R75231 and EHNA groups than the Control

group. Tissue blood flow after reperfusion was significantly better in the R75231 and EHNA groups than the Controls (Fig 1b). Histological analysis of post-ischemic liver tissue revealed that hepatocyte necrosis was similar among the EHNA and Control groups, but hepatocyte necrosis in the R75231 group was significantly lower than the other groups.

DISCUSSION

The effect of augmentation of endogenous adenosine was different based upon the inhibitor used. While inhibition of adenosine deaminase by EHNA significantly augmented adenine nucleotides and improved post-reperfusion blood flow, it did not significantly improve survival, liver function, nor liver histology. However, the augmentation of endogenous adenosine by the nucleoside transport inhibitor significantly improved survival, hepatic tissue blood flow, liver function, and liver histology.

There are several possible explanations for the difference in effectiveness among EHNA and R75231. First the inability of EHNA to protect the liver may be related to inhibition of other reactions, such as inhibition of dynein ATPase activity and actin dependent cellular processes.³ Second, the high adenosine levels caused by EHNA may induce systemic adverse events, including hypertension, renal vasoconstriction, and reduction of hepatic blood flow. Third, the high adenosine level induced by EHNA may stimulate purine catabolism and the production of superoxides through the xanthine oxidase system.⁴ A final explanation for the difference between R75231 and EHNA may be related to the mechanism of action of R75231. R75231 prevents the transport of adenosine from the hepatocyte and endothelial cells. It does not, however, prevent the transport of AMP from these cells. When AMP is transported into the interstitial space it is broken down to adenosine. The high adenosine levels in the interstitial

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Supported by Research Grants from the Veterans Administration and Project Grant No DK-29961 from the National Institutes of Health, Bethesda, Maryland.

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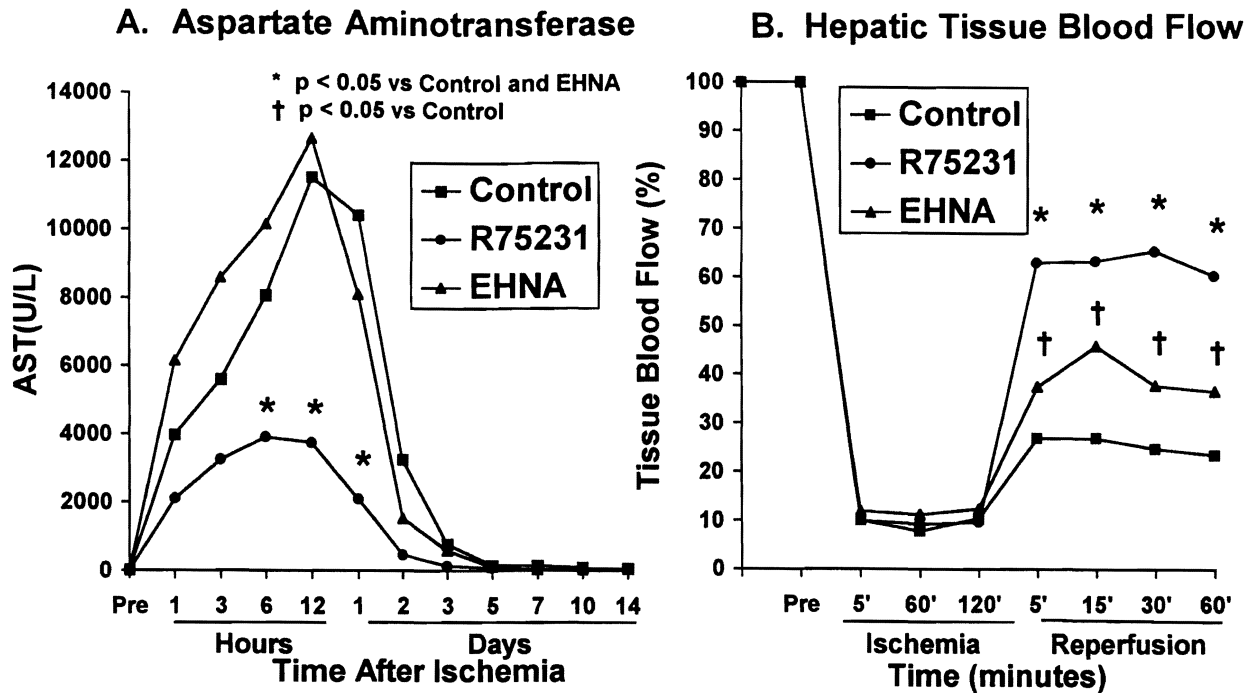


Fig 1. Liver function and hepatic tissue blood flow.

space may protect the hepatocyte and endothelial cells from ischemia and reperfusion injury.

Higher adenine nucleotide levels and adenosine levels in the ischemic liver was not associated with better post-ischemic liver function nor animal survival. Augmentation of endogenous adenosine by the nucleotide transport inhibitor, R75231, attenuated ischemia and reperfusion injury, while EHNA did not.

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