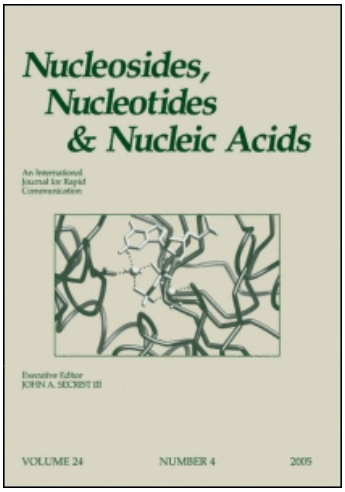

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Ecto-5'-Nucleotidase (Cd73)-Mediated Extracellular Adenosine Production Plays a Critical Role in Hepatic Fibrosis

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ECTO-5′-NUCLEOTIDASE (CD73)-MEDIATED EXTRACELLULAR ADENOSINE PRODUCTION PLAYS A CRITICAL ROLE IN HEPATIC FIBROSIS

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□ *In previous studies, we have demonstrated that adenosine and its receptors play a role in hepatic fibrosis. Here, we review evidence that toxin-induced increases in hepatic adenosine concentrations are generated from adenine nucleotides by the action of ecto-5′ nucleotidase and thus that adenosine-mediated, toxin-induced hepatic fibrosis depends on extracellular conversion of adenine nucleotides to adenosine.*

Keywords Adenosine; ecto-5′-nucleotidase; hepatic fibrosis; carbon tetrachloride; thioacetamide

INTRODUCTION

Extracellular adenosine arises from either an increase in intracellular adenosine, which is released into the extracellular space, or by the release of adenine nucleotides, which are dephosphorylated extracellularly to adenosine. Extracellular ATP and ADP can be converted into AMP by extracellular apyrases such as nucleoside triphosphate phosphohydrolase (CD39) and alkaline phosphatase, and AMP can then be converted into adenosine by ecto-5′-nucleotidase (CD73) or alkaline phosphatase.[1–3] Ethanol, viruses, drugs and metabolic derangements damage hepatocytes and stimulate fibrosis leading to cirrhosis. Ethanol is well known to stimulate increased extracellular adenosine concentration in vitro through its action on the nucleoside transporter, and ethanol ingestion increases purine release into the bloodstream and urine in normal volunteers.[4,5] In prior work, we

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have found that deletion or blockade of adenosine A_{2A} receptors prevents the development of hepatic fibrosis induced by TAA or CCL_4 indicating that extracellular adenosine plays a role in the pathophysiology of hepatic fibrosis.[6] However, the mechanisms governing adenosine formation in injured liver are not known, and the role of CD73 and extracellular formation of adenosine from adenine nucleotides has not been established in the liver. Here we review evidence that extracellular formation of adenosine by the action of CD73 plays a critical role in the development of hepatic fibrosis.

RESULTS

Extracellular Adenosine Concentrations Are Higher in the Supernates of Liver Slices from WT than CD73KO Mice

Hepatic slices from WT mice treated with PBS accumulated more supernatant adenosine than liver slices from CD73KO mice. As expected, after treatment with CCL_4, ethanol or TAA adenosine concentrations rose markedly in supernates of hepatic slices in both WT and CD73KO mice but much greater increases in adenosine concentration were observed in the supernates of WT liver slices than CD73KO mice. Thus, ecto-5′-nucleotidase plays an important but not exclusive role in extracellular adenosine formation in resting and toxin-challenged livers.

CD73KO Mice Are Resistant to CCL_4-Induced Hepatic Fibrosis

WT and CD73KO mice were treated with CCl_4 or vehicle (1.25 ml/kg CCL_4 in corn oil, 1:3 25% v/v or an equal amount of corn oil, biweekly intraperitoneal injections) for 6 weeks, and their livers harvested. The percentage of the total hepatic area that was picrosirius red-stained in vehicle-treated animals was $0.3 \pm 0.1\%$ and $0.3 \pm 0.1\%$ for both vehicle-treated WT and CD73KO mice ($n = 15$) whereas following CCL_4-treatment the livers of CD73KO mice were significantly less fibrotic ($P < 0.01$). The difference between the hydroxyproline content of livers from WT and CD73KO mice was highly significant ($P < 0.01$).

Adenosine Receptor mRNA Expression Increases After Chronic CCL_4 Treatment

Because extracellular adenosine appeared to play such an important role in CCL_4-mediated hepatic fibrosis we determined the effect of CCL_4 treatment on adenosine receptor expression and found that CCl_4 increased mRNA expression from 2- to 4-fold for all four of the adenosine receptors in both WT and CD73KO mice.

Col1α1, Col3α1 mRNA Expression Is Lower in CD73KO Mice

Hepatic fibrosis is characterized by extensive deposition of extracellular matrix (ECM) proteins, including collagen types I and III. Col1α1 and Col3α1 mRNA levels were nearly identical in vehicle-treated CD73KO mice and WT mice but increased significantly more in the livers of the CCl_4-treated WT mice than CD73KO mice.

Matrix Metalloproteinase, Tissue Inhibitor of Matrix Metalloproteinase mRNA Expression Increase After CCL_4 Treatment

In addition to increased synthesis and secretion, matrix degradation by proteases like the matrix metalloproteases (MMPs) also regulates the development of fibrosis. In the liver MMP-2, and MMP-14 play a role in extracellular matrix degradation and previous work from our laboratory has indicated that adenosine regulates MMP expression.[6] There was a marked increase in the expression of mRNA for all of these proteins after CCL_4 treatment.

DISCUSSION

Under resting conditions, adenosine is present at low concentrations in the extracellular space of most organs and adenosine levels rise substantially in response to hypoxia, tissue injury or metabolic stress. In previous work, we have demonstrated that CD73 is required for methotrexate- and H_2O_2-mediated adenosine release at a peripheral site of inflammation and that cultured human hepatoma cells (HepG2) and liver slices release more adenosine following in vivo challenge with toxins including ethanol, methotrexate and thioacetamide.[6,7] The results reviewed here indicate that CD73 plays an important role in adenosine generation in the liver and, consistent with prior studies, the extracellular adenosine generated by CD73 is a critical mediator of hepatic fibrosis induced by CCL_4.

We have previously demonstrated that adenosine A_{2A} receptors directly stimulate collagen production and that they play an active role in the pathogenesis of hepatic fibrosis.[6] Adenosine also induces hepatic stellate cell differentiation. The results described here are consistent with a role for extracellular adenosine in hepatic fibrosis.

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