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Effect of *S*-adenosyl-L-methionine on the activation, proliferation and contraction of hepatic stellate cells

Hiroko Matsui[a,b], Norifumi Kawada[c,*]

[a]*Department of Anatomy, Graduate School of Medicine, Osaka City University, Osaka, Japan*
[b]*Minophagen Pharmaceutical Co. Ltd., Tokyo, Japan*
[c]*Department of Hepatology, Graduate School of Medicine, Osaka City University, 1-4-3, Asahimachi, Abeno, Osaka 545-8585, Japan*

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Abstract

Inhibition of hepatic stellate cell activation is an important clinical aspect for the control of liver inflammation, fibrosis and cirrhosis. *S*-adenosyl-L-methionine (SAM), an intermediate product of L-methionine metabolism, is a precursor of glutathione and an endogenous methyl donor. Although the hepato-protective action of SAM has been reported in several animal models, the effect of SAM on the function of hepatic stellate cells has not been elucidated. Using a primary-culture model of hepatic stellate cells, we found that SAM blunts the activation process as indicated by the suppression of expression of collagen α1(I) and smooth muscle α-actin. SAM also hampers the DNA synthesis of hepatic stellate cells stimulated with a dimer of platelet-derived growth factor-B via the inhibition of phosphorylation of PDGF receptor-β and down-stream signaling pathways. SAM additionally inhibits the contraction of hepatic stellate cells by disturbing the formation of F-actin stress fibers and phosphorylated myosin light chains. Thus, SAM regulates the activation of hepatic stellate cells and may clinically contribute to therapy targeted at human liver fibrosis.

Keywords: Dimer of platelet-derived growth factor-B; Collagen; Smooth muscle α-actin; Endothelin-1; L-Methionine; L-Cysteine

1. Introduction

Hepatic stellate cells reside in sinusoids, maintain a quiescent phenotype, and store vitamin A (Okuyama et al., 2002; Eng and Friedman, 2000). In response to inflammatory stimuli, hepatic stellate cells undergo activation and proliferate, contract, secrete profibrogenic mediators, and generate extracellular matrix materials as well as play a role in the fibrosis of the liver (Friedman, 2000; Gabele et al., 2003; Pinzani and Marra, 2001). The suppression of hepatic stellate cell activation is thus a clinical issue which requires the establishment of therapeutic strategies against liver fibrosis (Albanis and Friedman, 2001; Bataller and Brenner, 2001).

Our previous studies have shown that L-cysteine, L-methionine and *N*-acetyl-L-cysteine exert anti-fibrotic activity in the liver (Kawada et al., 1998; Okuyama et al., 2001; Matsui et al., 2004). Oral supplementation of these amino acids suppressed septum formation and hepatic hydroxyproline content as well as mRNA expression of type I collagen, transforming growth factor beta (TGFβ) and tissue inhibitor of matrix metalloproteinases (TIMPs) in a thioacetamide-induced model of liver cirrhosis in rats. Mechanical analyses have confirmed that these amino acids directly affect the activation of primary-cultured hepatic stellate cells. Similar results were reported for different experimental conditions (Kim et al., 2001).

L-Methionine is metabolized to *S*-adenosyl-L-methionine (SAM) via methionine adenosyltransferase in the liver and in various extrahepatic organs (Avila et al., 2002; Lieber, 2002). In addition to its activity as a methyl donor, SAM has anti-inflammatory properties in the liver and is a precursor of glutathione. Thus it protects hepatocytes from oxidative stress (Lu et al., 2000, 2001; Martinez-Chantar et al., 2002;

* Corresponding author. Tel.: +81 6 6645 3811; fax: +81 6 6645 3813.
E-mail address: kawadanori@med.osaka-cu.ac.jp (N. Kawada).

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Halsted et al., 2002; Mato et al., 1999; Gasso et al., 1996; Varela-Moreiras et al., 1995). However, its action on constituent hepatic cells except for hepatocytes is largely unknown. Here, we report the inhibitory effect of SAM on the activation-associated function of primary cultured hepatic stellate cells.

2. Materials and methods

2.1. Materials

Collagenase and 3, 3′-diaminobenzidine tetrahydrochloride (DAB) were purchased from Wako Pure Chemical (Osaka, Japan). Pronase E was aquired from Merck (Damstadt, Germany), and recombinant rat dimer of platelet-derived growth factor-B (PDGF-BB) was obtained from R&D Systems (Minneapolis, MO). Polyclonal antibodies against extracellular signal-regulated kinases 1 and 2 (ERK1/2), phospho-ERK1/2 (Thr 202/Tyr 204), Akt and phospho-Akt (Ser 473) were purchased from Cell Signaling Technology, Inc. (Beverly, MA) and those against PDGF receptor-β, TGF-β receptor type II (TGFβRII), myosine light chain and phospho-myosine light chain were acquired from Santa Cruz Biotechnology (Santa Cruz, CA). Monoclonal antibody against smooth muscle α-actin, Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum, TRITC-labeled phalloidin and 5-bromo-2′-deoxyuridine (BrdU) were purchased from Sigma (Saint Louis, MO), and rat endothelin-1 was obtained from Peptide Institute (Osaka, Japan). Antibodies against STAP (stellate cell activation-associated protein) were generated in our laboratory as previously described (Kawada et al., 2001). Enhanced chemiluminescence detection reagent was purchased from Amersham Pharmacia Biotech (Buckinghamshire, England). A GeneAmp RNA polymerase chain reaction (PCR) Core Kit was obtained from PerkinElmer Life Sciences (Boston, MA), and isogen and agarose S were acquired from Nippon Gene (Tokyo, Japan). Immobilon P membranes were purchased from Millipore (Bedford, MA). Kodak XAR5 film was purchased from Eastman Kodak (Rochester, NY). All the other reagents were obtained from Sigma or Wako Pure Chemical.

2.2. Animals

Pathogen-free male Wistar rats were obtained from SLC (Shizuoka, Japan). The experimental protocols were approved by the Animal Research Committee of Osaka City University (Guide for Animal Experiments, Osaka City University).

2.3. Preparation of hepatic stellate cells

Hepatic stellate cells were isolated from male Wistar rats as previously described (Kristensen et al., 2000) and then cultured on plastic dishes in DMEM supplemented with 10% fetal bovine serum. After culturing had continued for the indicated number of days, the medium was replaced by serum-free DMEM with test agents and the culture was continued for 48 h.

2.4. BrdU incorporation assay

The incorporation of BrdU was immunocytochemically evaluated as previously described (Uyama et al., 2002). BrdU-positive cells were counted under four randomly chosen microscopic fields in each well. A BrdU labeling index (BrdU L.I.) was calculated as the number of BrdU-positive cells/number of cells in one area ×100 (%).

2.5. Immunoblot

Proteins were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and then transferred onto Immobilon P membranes. After blocking, the membranes were treated with primary antibodies followed by peroxidase-conjugated secondary antibodies. Immunoreactive bands were visualized using the enhanced chemiluminescence system (Amersham Pharmacia Biotech) and Kodak XAR5 film.

2.6. RT-PCR

mRNA expression was determined by reverse transcription-polymerase chain reaction (RT-PCR) using a GeneAmp RNA PCR Core Kit as previously described (Uyama et al., 2002). PCR products were then separated by electrophoresis on 2% agarose gels and stained with ethidium bromide. The primers used are listed in Table 1.

2.7. F-actin staining

F-actin in formalin-fixed cells was stained using TRITC-phalloidin as previously described and observed under a fluorescent microscope (Kawada et al., 1996a).

Table 1
Primer pairs used for RT-PCR in this study

	Forward	Reverse	Access no.
Smooth muscle α-actin	5′-TGTGCTGGACTCTGGAGATG-3′	5′-GATCACCTGCCCATCAGG-3′	X06801
Collagen α1(I)	5′-TGCCGTGACCTCAAGATGTG-3′	5′-CACAAGCGTGCTGTAGGTGA-3′	M11432
GAPDH	5′-GATGCTGGTGCTGAGTATGT-3′	5′-TCATTGAGAGCAATGCCAGC-3′	X02231