学 位 論 文 の 要 旨

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学 位 論 文 名	FR-167653, a Selective p38 MAPK Inhibitor, Exerts Salutary Effect on Liver Cirrhosis Through Downregulation of *Runx2*
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INTRODUCTION

Liver cirrhosis is recognized as a leading cause of significant morbidity and mortality worldwide and also poses an enormous healthcare problem in Japan because of its high mortality rate. Therefore, improved understanding of the underlying disease process and effective anti-fibrotic treatment regimens are needed urgently.

Myofibroblasts (MFs) including the hepatic stellate cells (HSCs) and portal fibroblasts are the major type of cells responsible for hepatic fibrosis. MFs are activated by multiple cytokines and growth factors including transforming growth factor-β (TGF-β and p38 mitogen-activated protein kinase (MAPK). An altered balance between the matrix degrading enzymes (matrix metalloproteinases; MMPs) and their inhibitors (tissue inhibitors of matrix protease; TIMPs) is also crucial for deposition of collagen fibers.

Several anti-cirrhotic agents have been developed over the past few years and it seems that most of them exert their effects by indirectly inhibiting the MAPK pathway in MFs. However, effect of a selective p38 inhibitor is yet to be reported. In this study, we evaluated the salutary effect of FR-167653 (FR), a selective p38 inhibitor, in a carbon tetrachloride

(CCl_4)-induced rat cirrhosis model.

MATERIALS AND METHODS

Twenty rats were assigned to four groups: (1) Sham; olive oil only, (2) Control; CCl_4 in olive oil, (3) FR50; FR 50mg/kg/day and CCl_4, and (4) FR100; FR 100mg/kg/day and CCl_4. Extent of hepatic fibrosis was determined by Azan-stained area and also by measuring hepatic hydroxyproline content. Activated MFs were detected by α-smooth muscle actin (SMA) immunostaining. Cellular expression of samd4 and TIMP-1 was evaluated by immunohistochemistry. Activation of p38 was assessed by the ratio of the phosphorylated and non-phosphorylated p38 with Western blot analysis. Expression of *runt-related transcription factor 2* (*runx2)* mRNA was assessed using RT-PCR. Quantification of the stained area was done by densitometric analysis of the stained area with the help of image analysis software.

RESULTS AND DISCUSSION

FR treatment significantly and dose-dependently reduced Azan-stained area and hydroxyproline content of the liver. Significant dose-dependent reduction in α-SMA immunostaining and improvement of the serum levels of AST, ALT and γGTP were noticed in the FR-treated rats. FR treatment might have produced salutary effect on liver cirrhosis by inactivation of MFs due to inhibition of release of inflammatory cytokines and inhibiting infiltration of inflammatory cells into the cirrhotic liver. Regarding the intracellular signaling pathway, FR inhibited activation of p38 and reduced Smad4 expression in MFs. These results implicate that the intracellular p38 is an essential downstream component of the fibrogenic signaling pathway. Moreover, mRNA expression of *runx2*, a profibrogenic transcription factor and downstream regulator of the p38 pathway, was significantly low in FR-treated livers. Further *in vitro* studies involving MFs isolated

from cirrhotic livers are necessary to prove the role *runx2* in cirrhosis. Reduced TIMP-1 expression in FR treated animals may indicate that FR prevents liver cirrhosis not only by inhibiting fibrogenesis but also by accelerating the fibrolysis. These date suggest FR may prove valuable in designing anti-fibrotic therapy and the ameliorating effect of FR could be partially attributable to an inhibition of the TGF-β/p38/*runx2*/TIMP-1 axis in MFs.

CONCLUSION

FR treatment exerted a significant beneficial effect in a CCl_4-induced rat cirrhosis model. The ameliorating effect of FR could be partially attributable to an inhibitory effect of the Smad4/p38/*runx2*/TIMP-1 axis in the cirrhotic liver. FR would be a new therapeutic option for the prevention and treatment of cirrhosis.