学 位 論 文 の 要 旨

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学 位 論 文 名　Exacerbation of Indomethacin-Induced Small Intestinal Injuries in *reg I*-Knockout Mice

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INTRODUCTION

Nonsteroidal anti-inflammatory drug (NSAID)-induced small intestinal injuries are serious clinical events and suitable therapeutic strategies have not been sufficiently established. Reg I (regenerating product I) is expressed in the gastrointestinal tract of various species and plays important roles in regeneration, proliferation, inflammation, and carcinogenesis. We previously found that Reg I is a potent regulator of cell growth that is required to generate and maintain the villous structure of the small intestine. These findings suggest that Reg I may exert protective effects in important for establishment of new therapeutic strategies for NSAID-induced small intestinal injuries based on its abilities to control cell proliferation, and maintain intercellular integrity and gut homeostasis. In the present study, we used Reg I-knockout (KO) mice to evaluate the role of Reg I and its therapeutic potential in NSAID-induced small intestinal injuries.

MATERIALS AND METHODS

First, we examined the time-course of changes in Reg I mRNA expression in the small intestines, and compared these with microscopic findings in order to clarify the pattern of Reg I expression in indomethacin-induced small intestinal injuries. Ten-week-old male ICR mice were injected

subcutaneously twice, with a 24-hour interval, with indomethacin at a dose of 80 mg/kg of body weight. The mice were euthanized at 3, 6, 12, or 24 hours after the last indomethacin injection, and Reg I mRNA expression were examined by real-time RT-PCR.

To examine the effect of Reg I deficiency on such injuries, indomethacin was injected subcutaneously into 10 week-old male Reg I-knockout (*Reg I*$^{-/-}$) and wild-type (*Reg I*$^{+/+}$) mice twice with an interval of 24 hours, after which the mice were euthanized. Small intestinal injuries were assessed by gross findings, histopathology, and contents of interleukin-1β (IL-1β) and myeloperoxidase (MPO) in the experimental tissues.

Next, we investigated the therapeutic potential of Reg I in indomethacin-induced small intestinal injuries. Recombinant Reg I protein (rReg I) was administered to 10-week-old male ICR mice, then indomethacin was administered 6 hours using the same protocol as noted above, after which small intestinal injuries were assessed after euthanasia.

RESULTS AND DISCUSSION

Reg I mRNA expression rapidly increased and peaked at 6 hours after the second indomethacin injection, followed by a gradual decline toward a normal level. Mucosal damage was maximal at 12 hours and histopathological examination showed development of small intestinal ulcers. These results indicate that after indomethacin administration, the expression of Reg I mRNA is upregulated in the early phase of small intestinal injuries, followed by the development of small intestinal ulcers. These results indicate that Reg I may also function as a protective factor in the small intestine, as well as in the stomach, against indomethacin-induced mucosal injuries.

In both *Reg I*$^{-/-}$ and *Reg I*$^{+/+}$ mice, administration of indomethacin provoked lesions in the small intestine. The length of the small intestine in the indomethacin-treated *Reg I*$^{-/-}$ mice was significantly shorter than that in the indomethacin-treated *Reg I*$^{+/+}$ mice. The area of macroscopically visible lesions in the *Reg I*$^{-/-}$ mice was significantly greater than that in the *Reg I*$^{+/+}$ mice at 24 hours after the last injection of indomethacin. Histological examinations revealed deep ulcers with prominent inflammatory cell infiltration and vascular thrombosis present.

However, this trend was more conspicuous in *Reg I*$^{-/-}$ mice. We further evaluated the expression of pro-inflammatory cytokines and MPO in the small intestine samples obtained from indomethacin-treated mice. Both IL-1β and MPO contents in the mucosa were significantly higher in *Reg I*$^{-/-}$ mice than in *Reg I*$^{+/+}$ mice. *Reg I*$^{-/-}$ mice developed more severe indomethacin-induced small intestinal injuries than *Reg I*$^{+/+}$ mice. These findings support our view that Reg I plays a protective role in indomethacin-induced small intestinal injuries.

Based on the above experiments using *Reg I*-KO mice, we speculated that rReg I administration might prevent indomethacin-induced small intestinal injuries. In order to test our speculation, we injected rReg I into the mouse model of indomethacin-induced small intestinal injuries. Although the small intestines of the experimental animals were shortened after the last indomethacin injection, this shortening in rReg I-treated mice was attenuated compared with vehicle-treated mice. The area of small intestinal injuries in the rReg I-treated mice was significantly smaller than that in the vehicle-treated mice. Microscopically, more shallow ulcers with less inflammatory cell infiltration were observed in the rReg I-treated mice. The mucosal content of IL-1β after indomethacin administration in the rReg I-treated mice was also significantly lower than that in the vehicle-treated mice. rReg I administration inhibited the development of indomethacin-induced small intestinal injuries. This therapeutic effect of rReg I was confirmed morphologically and biochemically. These results imply that Reg I may be useful as a therapeutic agent for indomethacin-induced small intestinal injuries.

CONCLUSION

The present results show for the first time that Reg I has a protective role against NSAID-induced small intestinal injuries and that rReg I effectively attenuates such injuries. Additional analysis of Reg I function in the small intestine may contribute to the development of a new therapeutic strategy for NSAID-induced small intestinal injuries.