Fullerenol C₆₀ for developing nanomedicine Part 2

- Assessment of therapeutic effect of fullerenol C₆₀ for inflammatory bowel disease -

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ABSTRACT SUMMARY

Inflammatory bowel disease (IBD) is an intractable disease that shows unexplained inflammation in the gastrointestinal tract. Although diverse therapeutic drugs were used, there were some problems with adverse effects and unresponsiveness. Therefore, the development of more effective medicine is highly required. Here, we focused on the polyhydroxylated fullerenes (fullerenols), C₆₀(OH)₃₆, because we previously showed that they had the strong anti-oxidative and anti-inflammatory effects in vitro. In this study, we investigated whether C₆₀(OH)₃₆ had a potential to be an useful nanomedicine for IBD. We administered C₆₀(OH)₃₆ to colitis model mice by dextran sulfate sodium (DSS), and measured the myeloperoxidase (MPO) activity, which is an index of colitis. As the result, the MPO activity was reduced by administration of C₆₀(OH)₃₆. Therefore, it is suggested that C₆₀(OH)₃₆ has a potential to be the effective nanomedicine for IBD.

INTRODUCTION

IBD, which is composed of ulcerative colitis and Crohn’s disease, is a chronic inflammatory disease of the gastrointestinal tract. IBD causes severe gastrointestinal symptoms, including diarrhea, abdominal pain, bleeding, anemia, and weight loss. It is thought that reactive oxygen species (ROS) is involved in initiating or propagating of the inflammatory process of IBD. Therefore, the therapeutic agents targeting ROS have been expected to prevent the IBD. Several anti-oxidative drugs have been used as the treatment for ulcerative colitis. However, some antioxidants, such as vitamin C, showed pro-oxidative effect under certain conditions, and the systematic alteration of the redox state may have adverse effects on the inflammatory response in certain disease states. Much effort is currently underway in searching for new effective antioxidants. In this regard, fullerenene C₆₀ is expected as a promising nanomedicine for the IBD, because fullerenene C₆₀ does not have the pro-oxidative effect and possesses the strong anti-oxidative effect. Therefore, we have focused on fullerenene C₆₀ as a new therapeutic agent for the IBD. In particular, we have used the water-soluble fullerenol C₆₀(OH)₃₆, because fullerenene C₆₀ showed a poor solubility in polar solutions. In our previous study, we evaluated the inhibitory effect of C₆₀(OH)₃₆ on the IL-1β-induced cytokine production in the human epithelial colorectal adenocarcinoma cell line (Caco-2 cells). As the result, C₆₀(OH)₃₆ significantly suppressed the level of IL-1β-induced cytokine production. Thus, it was indicated that C₆₀(OH)₃₆ possessed a strong anti-inflammatory effect.

In this study, we examined the therapeutic effects of C₆₀(OH)₃₆ in experimental models of IBD induced by dextran sulfate sodium (DSS).

EXPERIMENTAL METHODS

Colitis model: Female C57BL/6 mice were purchased from Nippon SLC (Kyoto, Japan) and used at 8 weeks of age. All mice except the non-treated group were treated with dextran sulfate sodium (DSS; 2.5%; molecular weight 36,000-50,000, MP biomedicals, LLC, Aurora, OH, USA) in their drinking water for 7 days. Mice were treated with C₆₀(OH)₃₆ at 25 mg/kg/day, 5-aminosalicylic acid (5-ASA) at 100 mg/kg/day, or distilled water (DW) by the intraperitoneal administration once a day for 7 days. Mice were sacrificed 12 h after the
last administration, and the colons were resected to determine the myeloperoxidase (MPO) activity.

**MPO assay:** The MPO activity was measured using a commercially available ELISA kit after homogenizing the colon. A mucosal scraping from the remnant colon was placed in 10 mg tissue/200 µL lysis buffer (200 mM NaCl, 5 mM EDTA, 10 mM tris, 10% glycerin, 1 mM PMSF, 1 µg/mL leupeptin, 28 µg/ml aprotinin, pH7.4). The solution was then homogenized and sonicated, and the resulting homogenate was centrifuged in a microfuge to remove any insoluble materials (15000 g for 15 min at 4°C), and the levels of MPO were measured.

**RESULTS AND DISCUSSION**

We examined the effects of fullerol C₆₀, C₆₀(OH)₃₆, on IBD in animal model of DSS. The DSS-induced colitis model has been extensively used because it shows similar symptoms compared to human colitis. We measured the levels of MPO activity as an index of acute inflammatory reaction with neutrophil infiltration into the colon. As a result, the MPO activity in DW-treated mice significantly increased on the day 7 after the DSS administration compared to that in non-treated mice. On the other hand, the MPO activity in C₆₀(OH)₃₆-treated and 5-ASA-treated mice significantly decreased compared to that in DW-treated mice (Figure 1). Therefore, it was indicated that C₆₀(OH)₃₆ had a potential to be a useful therapeutic nanomedicine for acute DSS experimental colitis. However, a significant weight loss by treating DSS was not improved in C₆₀(OH)₃₆-treated mice (data not shown). Thus, we are attempting to improve the therapeutic effect of C₆₀(OH)₃₆ by changing the route of administration such as rectum or oral route.

**CONCLUSION**

Fullerol C₆₀, C₆₀(OH)₃₆ has a potential to be an effective nanomedicine for IBD, although the safety assessment needs to be conducted as well. In the future, we will evaluate biodistribution and the stability of C₆₀(OH)₃₆ in order to identify the safety of C₆₀(OH)₃₆ as medicine. We hope that our studies could provide useful information for the development of innovative nanomedicines for IBD.

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![Graph](image-url)

**Figure 1.** Effect of C₆₀(OH)₃₆ on ulcerative colitis in mice by treating DSS. MPO activity was examined by MPO ELISA kit. Data are expressed as the mean ± S.E.M. (n=5-6). Represents significant difference from the non-treated group (**p < 0.01), and the DW-treated group (#p < 0.05) by Bonferroni.