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学位論文題目	**Development of an oral redox nanotherapeutics for treatment of colitis and colon cancer** 潰瘍性大腸炎と大腸癌の治療を目指した経口レドックスナノ粒子の開発

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論　文　の　要　旨

Inflammatory bowel disease (IBD), including **Crohn's** disease (CD) and **ulcerative colitis (UC),** affects millions of patients worldwide.[1] The intestinal mucosa of patients with IBD is characterized by reactive oxygen species (ROS) overproduction, leading to oxidative damage and imbalance in the constitution of intestinal microflora. Self-sustaining cycles of oxidant production may amplify inflammation and mucosal injury, promoting carcinogenesis.[2] In several experimental models, antioxidant compounds and free radical scavengers have improved colitis and inhibited inflammation-associated cancer development.[3] However, these low-molecular-weight (LMW) compounds are not completely effective due to a non-specific drug distribution, a low retention in the colon and severe adverse effects. If antioxidant compounds are specifically targeted to the diseased sites and effectively scavenge excessive generated ROS, they represent a safe and effective treatment for IBD. Nanoparticles such as liposome and polymeric micelles have gained worldwide attention as a new medical technology, because they change biodistribution of drugs to result in therapeutic effect of drugs significantly.[4] Recently, we have developed an amphiphilic block copolymer, poly(ethylene glycol)-*b*-poly[p-4-(2,2,6,6-tetramethylpiperidine-1-oxyl)aminomethylstyrene] (MeO-PEG-*b*-PMNT), possessing stable nitroxide radicals in the hydrophobic segment as a side chain via an amine linkage, which forms core-shell-type micelles in the physiological environment with an average diameter of about 40 nm, and termed nitroxide radical-containing nanoparticle (RNP^N).[5] Nitroxide radicals are confined in the core of this micelle, which shows high biocompatibility, including long-term blood circulation when administered intravenously and low toxicity. Therefore, RNP^N has been studied for therapy in oxidative stress injuries[5-9] and bioimaging.[10-11] For example, pH-sensitive RNP^N works effectively in acute renal injury[5] and cerebral ischemia-reperfusion[6] because it disintegrates in acidic conditions of diseased area by protonation of amino groups. However, pH-disintegrative character is not suitable for the treatment of IBD via oral administration.

In this study, a novel nanotherapy for the treatment of UC via oral administration is described. In order to target the nanoparticle to the colon area, its accumulation in the colonic mucosa is optimized, preventing its uptake into the bloodstream. A new designed redox polymer, methoxy-poly(ethylene

glycol)-*b*-poly[p-4-(2,2,6,6-tetramethylpiperidine-1-oxyl)oxymethylstyrene] (MeO-PEG-*b*-PMOT), is an amphiphilic block copolymer with stable nitroxide radicals in a hydrophobic segment as a side chain via an ether linkage and forms 40-nm-diameter core-shell-type micelles (RNP^O) by self-assembly in the aqueous environments regardless of pH . The objective of this study is to investigate the specific accumulation of RNP^O in inflamed colon and its therapeutic effects on colitis and colitis-associated colon cancer (CAC) mice models via oral administration.

The accumulation of nanoparticles in the colon area is one of the most important features for an effective nanomedicine against UC. In order to quantify the accumulation of nanoparticles in the colon area, we compared RNP^O with different sizes of commercial available polystyrene latex particles and LMW TEMPOL. Because we introduced nitroxide radicals into the particles, their accumulation could be quantitatively monitored by ESR measurements. When we orally administered LMW TEMPOL to mice, almost no ESR signal was observed in the colon. In contrast, polystyrene latex particles showed a higher accumulation in the colon compared to LMW TEMPOL with the size-dependent accumulation. Polystyrene latex particles with 40 nm and 100 nm in size accumulated higher than large-sized particles (0.5 μm and 1 μm). Interestingly, when RNP^O was administrated orally to mice, considerable high accumulation of RNP^O in colon was observed, as compared to polystyrene latex particles, even though the same size (40 nm). High colloidal stability of RNP^O due to the PEG tethered chains on the surface might be effective to accumulate in colonic mucosa as compared to polystyrene latex particles. In addition, the amount of accumulated RNP^O in the colon of colitis mice was 50% higher than that in the normal colon under the same administration conditions. Furthermore, no internalization of RNP^O in normal epithelial cells and no uptake of RNP^O into bloodstream were observed. This is in sharp contrast to LMW TEMPOL, which was absorbed into the bloodstream through the GI tract in normal mice and even more in colitis mice.[12]

Since orally administered RNP^O accumulated in the colonic mucosa of DSS-injured mice and was not absorbed into the bloodstream, it is anticipated to be an ideal nanomedicine for UC treatment. Therefore, the therapeutic and suppressive effects of RNP^O on dextran sodium sulfate (DSS)-induced colitis model mice were investigated. RNP^O was orally administered daily to DSS-injured mice for 7 days. Additional DSS-injured mice were treated with LMW TEMPOL, commercially anti-ulcer mesalamine and micelle without nitroxide radicals as controls. After 7 days of treatment, the mice treated with DSS had a significant increase in disease activity index (DAI), pro-inflammatory cytokine and ROS levels as compared to control mice. The treatments with LMW TEMPOL or mesalamine showed efficiency to decrease DAI, cytokine and ROS levels as compared to DSS-treated mice, though this efficiency was not significant. On the contrary, RNP^O-treated mice showed much lower inflammation compared to DSS-treated mice ($P < 0.01$) and other LMW drugs-treated mice. After 15 days of treatment, orally administered LMW TEMPOL and mesalamine slightly increased the survival rate (33.3% and 50%, respectively) compared with DSS- and micelle-treated mice (16.7%). On the other hand, RNP^O treatment significantly increased the survival rate of DSS-treated mice to 83.3%. These results indicate that RNP^O has not only suppressive but also therapeutic effects on mice with DSS-induced colitis.[12]

On the other hand, an imbalance in the constitution of intestinal microflora could lead to the dysregulation of host immunoreactivity towards intestinal bacteria, resulting in GI disorders and IBD.[13] Therefore, the effect of RNP^O on population of intestinal microflora was then evaluated. No remarkable differences in the total numbers of anaerobic and aerobic bacteria between experimental groups were observed during the DSS and RNP^O treatments. The changes in number of *E. coli* and *Staphylococcus* sp., commensal bacteria related to colitis, were also observed during treatment with RNP^O and DSS. The number of *E. coli* and *Staphylococcus* sp. significantly increases in DSS-treated mice, corresponding to the presence and severity of inflammation in colitis mice. In contrast, the number of these bacteria in mice treated with RNP^O and DSS was significantly reduced compared to DSS-treated mice ($P < 0.05$).[14] Importantly, the numbers of *E. coli* and *Staphylococcus* sp. in mice treated

with RNP^O alone were also similar to those of the control mice (10^4–10^6 CFU/g feces), indicating that oral administration of RNP^O did not affect the growth of these commensal bacteria. These results indicate that RNP^O did not affect at all to the microflora in normal mice and maintained the balance of microflora in colitis mice.[14]

Because RNP^O effectively suppressed the inflammation in colitis mice and did not disturb the intestinal microflora, the next challenge was to test the ability of RNP^O to CAC model, which is colon cancer driven from inflammation. Azoxymethane (AOM) and DSS were used to chemically induced CAC in mice. After 70 days treatment, AOM/DSS-treated mice displayed a significant increase in tumor score. However, the mice given 5 mg/mL RNP^O for a month had significantly reduced tumor scores compared to AOM/DSS-treated mice. Interestingly, when anticancer drug Irinotecan (Iri) was administered in combination with free drinking RNP^O, a remarkable suppression of tumor growth was observed in mice treated with combination compared to mice treated with Iri alone ($P < 0.05$). The Iri-induce adverse effects, such as diarrhea and GI toxicity, were remarkably reduced in RNP^O-treated mice ($P < 0.05$). These results indicate that oral administration of RNP^O not only significantly enhances the anticancer efficacy of Iri against CAC development, but also effectively suppresses the severe adverse effects of Iri.[15]

Based on the obtained results in this study, it is concluded that RNP^O is a very promising nanotherapeutics and drug delivery system for not only UC and CAC, but also other inflammation-associated diseases.

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審　　査　　の　　要　　旨

〔批評〕

論文審査及び質疑応答に関して、
本論文は活性酸素種を安全に効果的に消去するナノ粒子を経口投与型治療薬として用い、難治疾患で知られる潰瘍性大腸炎モデル、大腸バクテリアおよび大腸がん治療へと展開している。材料合成、薬物動態、治療メカニズムの解析投稿範囲にわたる研究を行い、副作用が無く、効果的に治療効果を出すことに成功した。このように本論文では新しい材料設計と機能構築にわたる広範な成果を上げている点で評価された。

〔最終試験結果〕

平成 27 年 2 月 19 日、数理物質科学研究科学位論文審査委員会において審査委員の全員出席のもと、著者に論文について説明を求め、関連事項につき質疑応答を行った。その結果、審査委員全員によって、合格と判定された。

〔結論〕

上記の論文審査ならびに最終試験の結果に基づき、著者は博士（工学）の学位を受けるに十分な資格を有するものと認める。