Catechol-Containing Antioxidant Micelles for Anti-Angiogenic Therapy

Masaki Moriyama¹, Stéphanie Metzger², Martin Ehrbar², André J. van der Vlies¹, Hiroshi Uyama¹, Urara Hasegawa¹

¹Graduate School of Engineering, Osaka University, 2-1 Yamadaoka, Suita, Osaka 565-0871, JAPAN; ²Department of Obstetrics, University Hospital Zurich, 8091 Zürich, SWITZERLAND
m-moriyama@chem.eng.osaka-u.ac.jp

ABSTRACT

The purpose of this study is to develop antioxidant polymeric micelles bearing catechol moieties. We prepared antioxidant micelles from poly(ethylene glycol)-b-poly(dopamine) (PEG-PDA) diblock copolymer. The diameter of the micelles was about 50 nm with narrow size distribution. These micelles efficiently scavenged ROS compared to the small antioxidant dopamine (DA). The micelles inhibited angiogenesis in HUVEC tube formation assay and the chicken chorioallantoic membrane (CAM) assay.

INTRODUCTION

Reactive oxygen species (ROS), such as hydrogen peroxide, the hydroxyl radical and superoxide anion, are continuously generated as byproducts of the metabolism of oxygen and play essential roles in cell signaling, pathogen defense and homeostasis. It is well known that overexpression of ROS is closely linked with initiation and progression of cancer, arthritis and other inflammatory diseases.¹ In addition, they are angiogenic mediators and promote angiogenesis which is essential for tumor growth and metastasis.² Therefore, ROS are now considered as therapeutic targets in many diseases including cancer.

Naturally-occurring antioxidants such as polyphenols, ascorbic acid and thiols are known to serve as safe ROS scavengers in the body. However, these molecules are generally susceptible to oxidation and readily lose their antioxidant activity under aerobic conditions resulting in low bioavailability and efficacy.

In the field of drug delivery, polymeric nanoparticles, generally in the range of 10-100 nm in dimension, are often used to deliver a wide variety of drugs including anti-cancer drugs and nucleic acids. They have been shown to prolong circulation time, improve biodistribution and minimize side effects of drugs.

Here we report polymeric micelles bearing catechol moieties with anti-oxidative activity. Their inhibitory effect on angiogenesis was also investigated.

EXPERIMENTAL METHODS

PEG-PDA diblock copolymers (Figure 1a) were synthesized by reversible addition-fragmentation chain transfer (RAFT) polymerization of N-acryloyl glycine tert-butyl ester using PEG-pyrrole carbodithioate as a chain transfer agent (CTA) followed by aminolysis to remove CTA end group, deprotection of tert-butyl ester group and conjugation of dopamine (DA) to introduce catechol moieties. The antioxidant micelles were prepared by dispersing PEG-PDA diblock copolymers in acetate buffer (pH 5.0). Micelle were characterized by dynamic light scattering (DLS) and transmission electron microscopy (TEM). To evaluate the oxidation stability under aerobic condition, DA and PEG-PDA micelles in air-saturated phosphate buffer saline (pH7.4) were kept at 37°C and tested for H₂O₂−scavenging activity.

To investigate whether DA and PEG-PDA micelles are able to exert an anti-angiogenic effect by scavenging endogenous ROS, we first evaluated their effects on the tube formation of vascular endothelial cells in vitro. Furthermore, we evaluated the anti-angiogenic activity of PEG-PDA micelles in the ex ovo chicken chorioallantoic membrane (CAM) assay.
RESULTS AND DISCUSSION

DLS showed that PEG-PDA polymer formed micelles with narrow size distribution. The diameter of the micelles was 50 nm. The TEM image showed the spherical shape of the micelles (Figure 1B).

As shown in Figure 1C, freshly prepared solutions of DA and PEG-PDA micelles (incubation time: 0 h) scavenged 80-90% of the added H₂O₂. In the case of DA, the H₂O₂-scavenging activity was reduced as function of incubation time. The scavenged H₂O₂ was 50% after 48 h. In addition, we observed a dark-brown precipitate after 48 h incubation. Contrary to DA, PEG-PDA micelles kept their H₂O₂-scavenging activity after 48 h of incubation.

As shown in Figure 2, the non-treated cells formed capillary-like structures (arrows). On the other hand, the tube formation capacity was impaired upon the addition of PEG-PDA micelles. To confirm that the inhibition of the tube formation was due to the ROS-scavenging activity of the micelles, the intracellular ROS level in HUVECs treated with DA and PEG-PDA micelles was visualized using a ROS-detection dye, dichlorofluorescin-diacetate (DCFH-DA). In the case of PEG-PDA micelles, the fluorescence due to the intracellular ROS was not observed while DA slightly diminished the intracellular ROS level compared to the non-treated cells. These results indicate that the long-lasting ROS-scavenging activity is critical to exert an anti-angiogenic effect.

CONCLUSION

We prepared polymeric micelles from PEG-PDA diblock copolymers. The micelles showed high oxidation stability under aerobic condition. In the tube formation assay and the CAM assay, the micelles exerted anti-angiogenic activity. These results suggest that the antioxidant micelles containing catechol moieties may be useful in anti-angiogenic therapy to treat many diseases including cancer.

REFERENCES

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