Study on interaction between plasmid DNA and berberine derivatives with aliphatic chain by fluorescence analysis

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ABSTRACT

In this study, the fluorescence analysis was used to reveal the interaction between berberine derivatives and plasmid DNA. The results showed that berberine (C0) and its 8-alkyl derivatives can enhance the fluorescent intensity of plasmid DNA. Compared with 8-dodecyl- (C12) and 8-hexadecyl- (C16) berberine, 8-alkylberberine with shorter alkyl group, such as 8-ethyl (C2), 8-butyl (C4), 8-hexyl (C6), and 8-octyl (C8) berberine derivatives showed higher fluorescence increasing effect. Among all compounds, C4 showed highest fluorescence increasing effect. All compounds tested obviously enhanced fluorescent intensity at the concentration of 6.25×10^{-5} mol/L. These results suggested that berberine and its derivatives can be selectively inserted to the grooves running down the plasmid DNA helix, thus, lead to the increase of fluorescence intensity of the reaction system. Also, adding proper length of aliphatic chain to berberine could promote the interaction between DNA and berberine derivatives. The results of this study may lay some useful foundation for the development of berberine-based medicine agents.

Key words: Berberine, berberine derivatives, fluorescence analysis, interaction, modify with alkyl group, plasmid DNA

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INTRODUCTION

Studying the interaction between medicinal agents and biological molecule is of great importance to understand the pharmacology mechanism and potential side effects of the candidate drugs. Berberine (BBR) is a natural alkaloid isolated from Huanglian (*Coptis chinensis Franch*) and used in the treatment of various diseases due to its significant anti-diabetic, hyperlipidemia, antibacterial, anticancer, and antiviral effects.[1-3] Studies have demonstrated that BBR could strongly interact with biological samples by ultraviolet (UV) and fluorescence methods.[4-6] For instance, He *et al*., reported that BBR can intercalate at the DNA grooves and enhance its fluorescence intensity,[5] and Wang *et al*. proposed that hydrophobic interaction was one of the binding force.[7] Previous study also suggested antimicrobial activity of BBR derivatives substituted with alkyl chain increased as the length of aliphatic chain was elongated and then decreased gradually when the alkyl chain exceeded 8 carbon atoms.[8,9] In order to investigate the structure–activity relationship of BBR derivatives and their antimicrobial mechanism, it is of great importance to determine the influence of hydrophobicity on the binding affinity of DNA and BBR, which was modified with different alkyl chain. In this study, aliphatic groups with different lengths were added to BBR to evaluate their interactions with DNA. The results of this study may lay some useful foundation for the development of BBR-based medicine agents.

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MATERIALS AND METHODS

In all, 8-ethyl (C2), 8-butyl (C4), 8-hexyl (C6), 8-octyl (C8), 8-dodecyl (C12), and 8-hexadecyl (C16) BBR derivatives were synthesized according to a previous study;[7] all compounds were of purity above 98% (analyzed by HPLC). Bacterial plasmid was provided by School of Pharmaceutical of Southwest University (A260/A280 = 1.87, which implied that the nucleic acid was effectively pure).

BBR (C0) and its derivatives was diluted in the preparation of phosphate buffer solution (pH 7.0) to the following concentrations: 2.5×10^{-4}, 6.25×10^{-5}, 1.56×10^{-5}, and 3.9×10^{-6} mol/L. Phosphate buffer solution (pH 7.4) 1% DNA suspension was prepared in phosphate-buffered saline (pH 7.4).

All experiments were performed with triplicates. Briefly, 0.5 mL of 5% plasmid DNA was added to 1.5 mL of BBR and its derivatives with different concentrations (2.5×10^{-4}, 6.25×10^{-55}, 1.56×10^{-5}, 3.9×10^{-6}, and 0 mol/L). After the reaction mixture was incubated at 37°C for 5 min, the fluorescent intensity was recorded on an F-4500 fluorescence spectrophotometer (Hitachi Company, Japan) at 368 nm excitation and 530 nm emission when both of the slit widths of excitation and emission raster were 5 nm.

RESULTS

As shown in Figure 1, plasmid DNA only emitted little fluorescence, the addition of BBR and BBR derivatives strongly increased the fluorescence intensity of the reaction system. Among all samples, 8-butyl-BBR (C4) exhibited the highest fluorescence intensity, while the 8-hexadecyl BBR (C16) showed the lowest. Meanwhile, at the range of 0 to 6.25×10^{-5} mol/L, the fluorescence intensity was enhanced with the concentration of each compound increasing. Interestingly, when the concentration of BBR and its derivatives exceeded 6.25×10^{-5} mol/L, the fluorescence was decreased, except for 8-hexadecyl-BBR (C16). Unlike the fluorescence probe EB, which could insert into the base pairs of DNA,[10] BBR and its derivatives could bind to the grooves that run down the plasmid DNA helix, causing an increase of the fluorescent intensity.[5]

To compare the DNA-binding activity of BBR derivatives, 0.5 mL plasmid DNA were added to 1.5 mL (6.25×10^{-5} mol/L) of BBR (C0) and C2, C4, C6, C8, C12, and C16 modified derivatives and then recorded the change in the fluorescence intensity. As shown in Figure 2, aliphatic modification changed fluorescence emission in the reaction system to different degree. In total, C2, C4, C6, and C8 promoted the fluorescence intensity, especially for C4, which was twice as much as BBR. However, C12 and C16 sharply decreased it as compared with BBR. These data suggested that proper aliphatic chain was beneficial to the interaction between DNA and BBR derivatives, but too long a length can reduce the interaction, such as that between C12 and C16.

A total of 1.5 mL of BBR derivatives with different concentrations were added to the reaction system and the fluorescence intensity were recorded to evaluate the interaction of these compounds and plasmid DNA. As shown in Figure 3, fluorescence enhanced with increasing concentration of compounds. When their concentration exceeded 6.25×10^{-5} mol/L, the fluorescent intensity was decreased, except for C16. These results indicated that BBR and its derivatives can selectively inserted to the grooves that running down the plasmid DNA helix, adding proper length of aliphatic chain enhanced the interaction between DNA and BBR derivatives (C2, C4, C6, and C8). At 6.25×10^{-5} mol/L, compounds (C0, C2, C4, C6, C8, and C12) could increase the fluorescence in the reaction system, and each group reached its maximum fluorescence intensity. These results indicated that adding different length of aliphatic chain to BBR has no influence on the binding site of BBR derivatives and DNA.

However, C12 and C16 showed relatively weaker activity of enhancing fluorescence, and C16 exhibited difference rule to other compounds, as the fluorescence intensity of C16 group was enhanced slowly with the increase of its concentration in test concentration. This result indicated that adding large aliphatic chain to BBR would go against the interaction of BBR derivative and plasma DNA.

DISCUSSIONS

A variety of pharmacological action of BBR is confirmed by different experiment, but the explicit mechanism or therapeutic targets for BBR remedying disease need further investigation. Yi *et al.* evaluated the antimicrobial mode of BBR by LC/ESI-MS combined with principal component analysis, their results indicated that the antimicrobial mechanism of BBR was similar to that of the most effective antimicrobial drug: Rifampicin and norfloxacin.[11] Thus, they concluded the antimicrobial target of BBR was nucleic acid. In our previous study, we found that among BBR and its derivative, C8, showed the highest antimicrobial activity *in vitro*. However, in this study, the fluorescence of C4 group was highest, which means the strongest interaction between C4 and DNA, and C8 did not exhibit the highest interaction with DNA. This result is inconsistent with the rule of antimicrobial activity of BBR derivatives and indicated that DNA is may not the potential target for antimicrobial therapy. In other words, BBR and its derivatives possess different

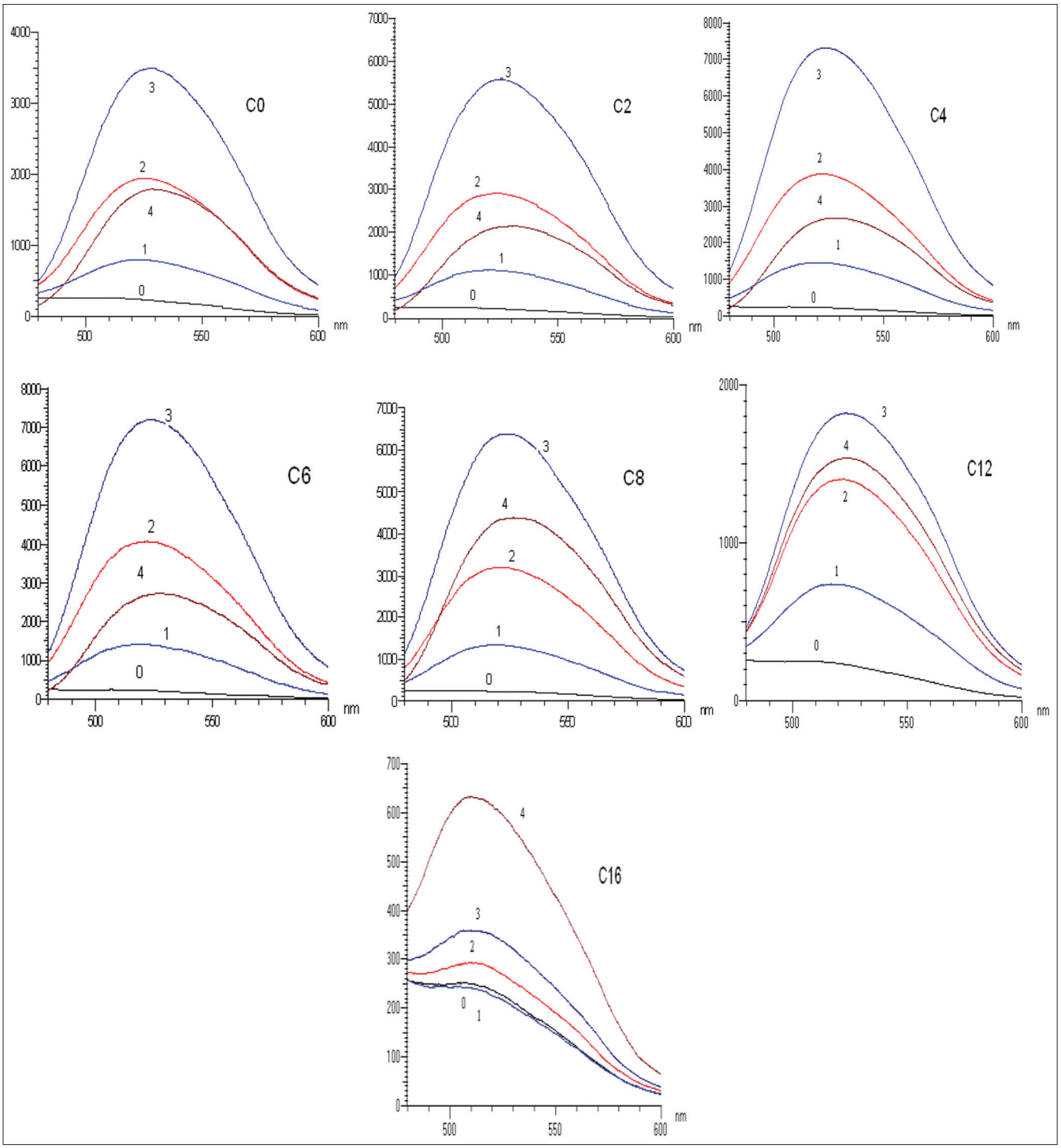

Figure 1: The fluorescence intensity of plasmid DNA under berberine derivatives with different concentration. 0–0 mol/L; 1–3.9 × 10^{-6} mol/L; 2–1.56 × 10^{-5} mol/L; 3–6.25 × 10^{-5} mol/L; 4–2.5 × 10^{-4} mol/L.

antimicrobial mechanism from other antimicrobial drug and are good candidate for the new drug research. Since the results obtained by this study demonstrated the strong affinity between nucleic acid and BBR derivatives and DNA mutation plays a crucial role in the process of cancer, the underlying mechanisms of DNA binding activity and anticancer ability of BBR derivatives should be studied further.

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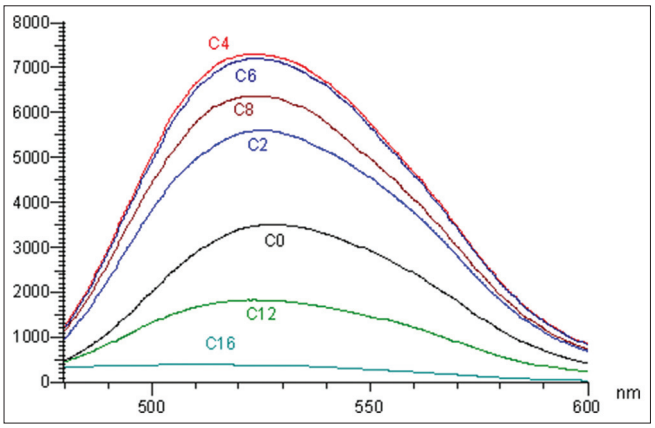

Figure 2: The fluorescence spectrum of berberine derivatives reacting with DNA at the concentration of 6.25 × 10^{-5} mol/L

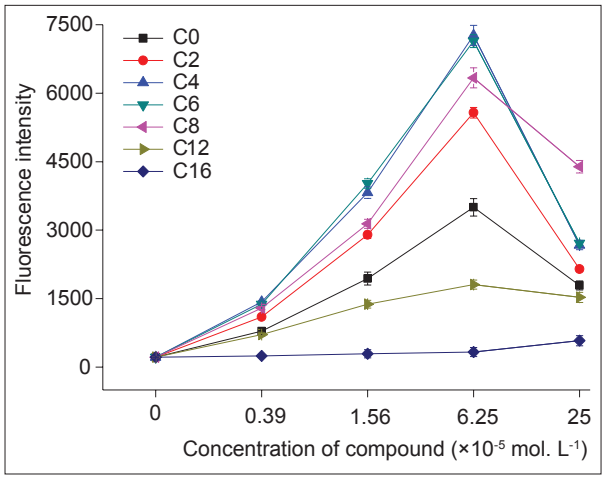

Figure 3: Effects of berberine derivatives on fluorescence intensity of plasmid DNA

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