DOCTORAL THESIS

The noncovalent binding of benzophenathridine alkaloids to double-stranded, bulged and G-quadruplex DNA

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Date of Award:
2008

The Noncovalent Binding of Benzophenanthridine Alkaloids to Double-stranded, Bulged and G-quadruplex DNA

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A thesis submitted in partial fulfillment of the requirements

for the degree of

Doctor of Philosophy

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Hong Kong Baptist University

September 2008

ABSTRACT

Quaternary benzophenanthridine alkaloids (QBAs) sanguinarine, chelerythrine and nitidine, are a class of pharmacologically-active constituents of some Chinese herbal medicines, such as Herba Chelidonii (白屈菜), Radix Zanthoxyli (两面针) and Herba Macleayae (博落回). As strong anti-bacterial agents, they are being used in oral hygiene products to improve gingival health. QBAs possess diverse pharmacological activities including potent antitumor, promising antimicrobial, apoptosis induction, and antiviral activities. However, the molecular mechanisms of their pharmacological actions remain unclear.

This research project mainly focused on the binding capacity and sequence selectivity of QBA representatives to double-stranded (ds) DNA, site-specificity and bulge base selectivity to functional bulged DNA, and binding properties to human telomeric G-quadruplex DNA, which has emerged as a significant and valid target for the development of new generation of anticancer drugs. To achieve these goals, various analytical techniques including UV-VIS absorption, fluorescence spectrometric titration, CD spectrometry, DNA polymerase stop assay, UV melting, ESI-TOF-MS and competitive ethidium bromide (EB) displacement assay were combinatively employed to comprehensively investigate the DNA-binding activities of anticancer QBAs. The results are summarized as follows:

(1) Sanguinarine, chelerythrine and nitidine were demonstrated to be double-stranded DNA intercalators not only with extremely strong binding affinity, but also with distinct sequence selectivity. Both sanguinarine and nitidine bind preferentially to DNA containing alternating GC base pairs. While chelerythrine showed a striking specificity for DNA containing contiguous GC base pairs, exhibiting quite distinct sequence selectivity from sanguinarine. Interestingly, nitidine also exhibited insecondary selectivity to the contiguous GC sequence.

(2) Chelerythrine and sanguinarine were found to specifically recognize bulge sites in DNA hairpins, which is irrelevant to the base composition of hairpin loop and stem. Two alkaloids can even specifically differentiate the type and number of base in the bulge site of DNA hairpins. Both alkaloids followed an identical tendency of binding selectivity to single-base bulge sites, *i.e.* C-bulge > T-bulge >> G-bulge = A-bulge, revealing a significant preference for binding site of single pyrimidine (C, T) bulge over the purine bulge (A, G). However, sanguinarine and chelerythrine showed different behaviours in binding to bulged DNA. In regard to binding ability, the more planar sanguinarine binds to both regular and bulge hairpins more strongly than chelerythrine, but chelerythrine exhibited much higher binding selectivity toward bulge hairpins than sanguinarine.

(3) Sanguinarine and chelerythrine specifically bind to and stabilize human telomeric K^+-form G-quadruplex DNA. In sharp contrast, these two alkaloids exhibited no significant stabilizing effect on the Na^+-form G-quadruplex DNA. Both sanguinarine and chelerythrine can further induce the formation of G-quadruplex. The conformation of intramolecular G-quadruplex induced from the random coil of human telomeric sequence by QBAs was identified to be K^+-form, which is the unique characteristic of G-quadruplex-interactive compounds.

(4) "Beads-on-a-String" structure in the human telomere proposed by Yu *et al.* was confirmed in this research by studying the binding behaviours of sanguinarine to long human telomeric sequence. Upon the verification of "Beads-on-a-String" structure, an unprecedented mode of sanguinarine-induced stacking of G-quadruplex beads in which sanguinarine favourably binds between tandem G-quadruplex beads was proposed. Sanguinarine binding to telomeric DNA in such a mode could inhibit telomerase activity more effectively.

(5) The differences between sanguinarine and chelerythrine in binding to human telomeric G-quadruplex DNA are significant. With respect to binding ability, sanguinarine showed much stronger binding and stabilizing activity to K^+-form G-quadruplex DNA than chelerythrine. With regard to G-quadruplex inducing ability, sanguinarine also exhibited more potent induction capacity than chelerythrine.

In conclusion, these findings shed lights on the molecular mechanism for diverse pharmacological activities of QBAs, and advanced insights into DNA-binding activities of anticancer QBAs. The strong binding ability of three QBAs toward double-stranded DNA may be partially responsible for their cytotoxicity to cancer cells. Additionally, the large DNA-binding affinities of QBAs alert the public to the safety concern of daily-used hygiene products and Chinese herbal medicines containing these alkaloids.

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