Detection of Antibacterial Activity of Berberine Hydrochloride by Multiwalled Carbon Nanotubes

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In order to investigate the effect of multiwalled carbon nanotubes (MWCNTs) as a carrier of drug on its cargoes, this paper choose berberine hydrochloride (BH) as a model and find that MWCNTs have the ability to enhance the antibacterial activity of BH.

Novel nanomaterials such as quantum dots,[1] colloidal nanoparticles,[2] nanowires,[3] nanohorns,[4] and nanotubes[5,6] have been extensively studied and applied in every aspects in science research and nature explorations. As one of the most exciting nanomaterials, carbon nanotubes (CNTs) discovered in 1991,[7] have been employed frequently in electrochemistry because of their remarkable electronic, thermal properties, and great strength, rigidity.[7] On the other hand, CNTs could be applied widely in biological fields after their solubility in aqueous medium has been resolved, especially in biomedical and biotechnology including biomolecules immobilization (e.g., enzyme, nucleic acids, protein, antigen), biosensors,[8] gene therapy,[9,10] drug delivery,[11–13] and tissue supports.[11]

Being biomolecule transporters, CNTs at least have two advantages. Firstly, CNTs can get across the cell membranes easily, which is essential in biological processes. Secondly, CNTs have strong near-infrared absorption, in which spectral region biological systems are highly transparent.[14,15] So CNTs can be used as optical stimulators in living cells to destruct target cells, avoiding the damage of normal cells.

Acting as biomolecule transporters, CNTs' toxicity and biocompatibility have been investigated,[12,13] and it was found that CNTs are less toxic to biological systems compared with other nanomaterials such as quantum dots and nanofibers. However, the potential influences of CNTs on the cargos in cargo–carrier systems, especially in drug–carbon nanotube systems, are still remained uninvolved. On this aspect, we believe that the influences of multiwalled carbon nanotubes (MWCNTs) to their cargos are very critic to estimate the effect of drugs since the CNTs are exotic in these systems. Therefore, we employ berberine hydrochloride (BH) and acid-treated MWCNT system as the models to estimate the biological significance. Our results showed that antibacterial effect of BH could be enhanced if transported by MWCNTs, which might be demonstrated by the measurements of flow cytometry with propidium iodide (PI) indicator. PI can enter into cell interior, intercalate into DNA through the minor groove, and emit strong fluorescence when the intact cell membranes are destroyed.[13] Therefore, PI is a membrane-impermeable dye, and generally employed as an indicator of cell death or the cell viability with the percentage of injured or dead cells.[16] Generally, the intensity of PI represents the injured degree of cell membranes, and the percentage of it represents the number of injured or killed cells.

In order to assess the enhancing antibacterial activity of BH by MWCNTs, we should at first measure the toxicity of MWCNTs. In our experiments MWCNTs scarcely display toxicity to *E. coli* when the concentration of MWCNTs is lower than 4.8 μg/mL, while gradually become toxic when higher than that concentration (see Supporting Information). On the other hand, the effect of BH–MWCNT complexes shows that the killed or injured cells became maximum value when the concentration of MWCNTs is 3.6 μg/mL, indicating that the strongest enhancing antibacterial effect of MWCNTs to BH.

To obtain the optimal enhancing antibacterial effect of BH by MWCNTs, we co-cultured BH and MWCNTs with *E. Coli*, which were in logarithmic phase, drug free and BH-treated *E. coli* were used as controls. Samples were measured by flow cytometry using PI fluorescence. It was found that the fluorescence intensity and percentage of PI dyes have no dependence on culture time for drug free control in our experiment (Figures 1A–1E shaded region), bacterium treated with BH and BH–MWCNT complexes have no significant changes on the fluorescence intensity (Figure 1, thick and dotted lines). When

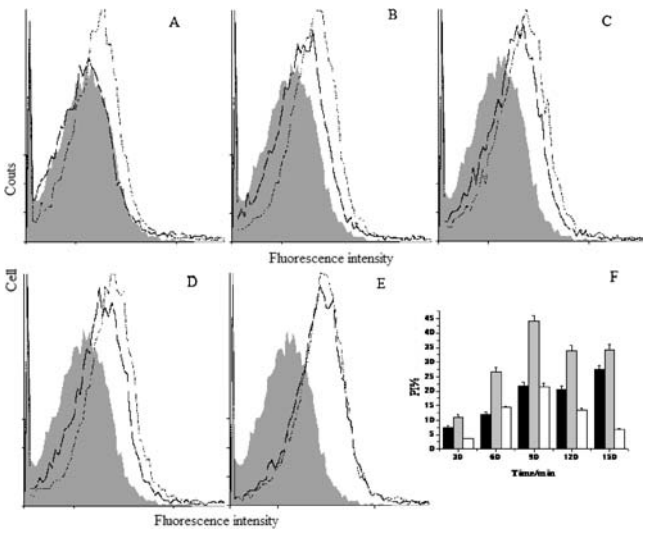

Figure 1. Fluorescence emission of *E. coli* inoculated with BH or BH–MWCNT complexes. Concentrations of BH and MWCNTs are 40.79 mg/mL and 3.6 μg/mL, respectively. Shaded region represents the fluorescence intensity of PI with drug free, and each thick and dotted line represents the fluorescence intensity of PI with BH and BH–MWCNTs. (A) to (E) display the inoculation results of 30, 60, 90, 120, and 150 min. (F) displays PI percentages of *E. Coli*, where black and gray pillars are the cells inoculated with BH and with BH–MWCNT complexes. White pillars represent the differences of percentage of PI between the region of gray and black.

treated with BH (Figure 1F, black pillars), the percentage of PI increasing with culture time. For bacterium treated with BH–MWCNT complexes, the percentage increased gradually in the first 90 min, then decreased, and became steady at 150 min (Figure 1F, gray pillars). As the white pillars showed in Figure 1F, the percentage of PI enhanced most after inoculation for 90 min, demonstrating that MWCNTs have maximum enhancing antibacterial effect to BH when inoculated *E. coli* with BH–MWCNT complexes for 90 min.

Comparing the PI percentage of *E. Coli* inoculated with BH–MWCNT complex with that with BH in different concentrations of BH, we can find that the enhancing antibacterial effect depends on BH concentration. The PI different between that treated with BH–MWCNTs and that with BH are 1, 17.1, and 8.8%, correspond to BH concentration of 163.44, 40.79, and 16.34 mg/mL, showing that MWCNTs have the ability to enhance antibacterial effect of BH, especially when BH is close to minimum inhibitor concentration. When BH is in higher than minimum inhibitor concentration, BH alone can kill *E. coli*, and MWCNTs here is dispensable. However, when the concentration is lower than minimum inhibitor concentration, BH just inhibits *E. coli*. The addition of MWCNTs is indispensable for its antibacterial activity and the enhancing antibacterial effect of MWCNTs stands out, this is highlight when BH is in close to it's minimum inhibitor concentration.

About the mechanism concerning the enhanced antibacterial activity of BH by MWCNTs we speculate BH can enter into cell membranes easily through the bridge effect of MWCNTs, because MWCNTs have high affinity to cell membranes and BH. The affinity of MWCNTs to cell membranes is observed by transmission electron microscopic (TEM), and the interaction of BH and MWCNTs is displayed by FT-IR. Washed and resuspended MWCNTs (left), bacteria treated with BH–MWCNT complexes (middle), and BH (right) are shown in Figure 2A. Bacterial treated with BH–MWCNT complexes are black whereas MWCNTs are colorless, and bacterial treated with BH are buff attributable to the color of BH. After washing, MWCNTs were in superstratum and discarded, but when concurrence with bacteria, it is in substratum. The phenomena suggesting that MWCNTs may absorb on bacterial membranes or enter into bacteria, further confirmation was done by TEM.

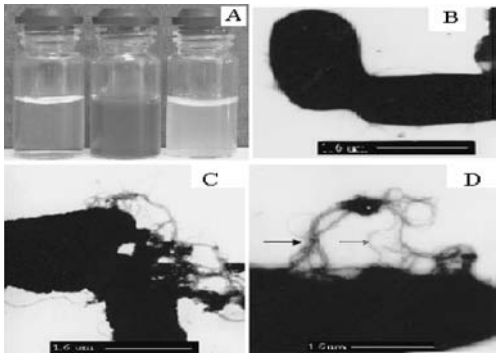

Figure 2. The affinity of MWCNTs to *E. Coli*. (A) The resuspensions of MWCNTs (left), *E. Coli* treated with BH–MWCNTs complexes (middle) and BH (right). TEM images of *E. Coli* (B), *E. Coli* treated with MWCNTs (C), and BH–MWCNT complexes (D), respectively.

Compared with bacteria treated with BH except flagellum of *E. Coli* (Figure 2C broken line arrow), MWCNTs being thick and out-of-order tubes have seen in bacteria treated with MWCNTs and BH–MWCNT complexes (see in Figure 2C and Figure 2D). On the other hand, BH contains multibenzene rings which can absorb on the surface of MWCNTs by π–π interaction.[17] This can be also confirmed by IR spectra (see Supporting Information), first, the vibration of benzene ring (1600.38 and 1505.04 cm^{-1}) disappeared in BH–MWCNT complexes. Second, BH–MWCNT complexes show peaks at 1396.54 and 1110.51 cm^{-1} (appearing at 1392.09 and 1105.36 cm^{-1} in BH), which has the similar phenomena to the interaction of CNTs with tetra-*tert*-butylphthalocyanines.[18]

In summary, we have developed a drug-carrier system to study the influence of carrier to cargos by flow cytometry, TEM and FT-IR measurements, and the enhancing antibacterial activity of BH by MWCNTs has been confirmed. When BH, MWCNTs, and bacteria coexist in solution, BH can enter into bacterial membranes easily through the bridge effect of MWCNTs, as a result, the antibacterial effect of BH enhanced.

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