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Cytotoxic activities of chitosan nanoparticles and copper-loaded nanoparticles

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Abstract—Chitosan nanoparticles and copper(II)-loaded chitosan nanoparticles were prepared based on the ionic gelation of chitosan with tripolyphosphate anions and copper ion sorption. In this study, the cytotoxic activities of the chitosan nanoparticles and copper(II)-loaded chitosan nanoparticles was investigated and a relationship between physiochemical properties and activity is suggested. The chitosan nanoparticles and copper(II)-loaded chitosan nanoparticles elicited dose-dependent inhibitory effects on the proliferation of tumor cell lines.

Chitosan is a cationic polysaccharide made from alkaline N-deacetylation of chitin. It has attracted much attention as a biomedical material, owing to its unique biological activities including antitumor activities, immuno-enhancing effects, increased protective effects against infection with some pathogens,[1] antifungal, and antimicrobial activities.[2,3] Chitosan could be developed as sole drugs for its biological activities. Cytotoxicity of chitosan and its derivatives toward tumor cells were previously studied.[4,5] Chitosan nanoparticles have been synthesized as drug carriers as reported in previous studies.[6–8] However, the cytotoxic activity of chitosan nanoparticles has only seldom been reported elsewhere. In our previous reports, chitosan nanoparticles and copper(II)-loaded nanoparticles with various mean particle size and surface charge had been prepared and characterized to investigate their heavy metal sorption and antibacterial activities. It showed that chitosan nanoparticles with little particle size and positive surface charge could exhibit higher sorption capacity and antibacterial activity.[9,10] The unique character of chitosan nanoparticles could provide higher affinity with negatively charged biological membranes and site-specific targeting in vivo.[9] In this study, the cytotoxic activities of the chitosan nanoparticles and copper(II)-loaded chitosan nanoparticles was investigated and a relationship between physiochemical properties and activity is suggested.

Chitosan nanoparticles with mean particle size ranging from 40 to 100 nm and positive surface charge about 50 mV, and copper(II)-loaded chitosan nanoparticles with mean particle size of about 257 nm and positive surface charge of about 96 mV were prepared and characterized in our previous reports.[9,10] Chitosan nanoparticles and copper(II)-loaded chitosan nanoparticles were filtered by membrane with diameter 0.45 μm and autoclaved to remove any contaminant before used in cell culture. The obtained nanoparticles were stable under the autoclaving conditions.[9]

BEL7402, BGC823, Colo320, L-02 cell lines were obtained from the Cell Bank of the Chinese Academy of Science, Shanghai, China. The cell line was cultured in RPMI-1640 supplemented with 10% heat-inactivated fetal bovine serum (GIBCO). The tumor cells were cultured for 24 h in a 96-well plate (Corning, America) at an initial concentration of 5×10^4 cells, then the cells were immediately treated with various doses (25–100 μg/mL) of chitosan nanoparticles for another 24 h. Viable cells were identified and counted using the tryphan blue dye exclusion test.

The results presented here suggest that chitosan nanoparticles and copper(II)-loaded chitosan nanoparticles showed much higher cytotoxicity than chitosan toward

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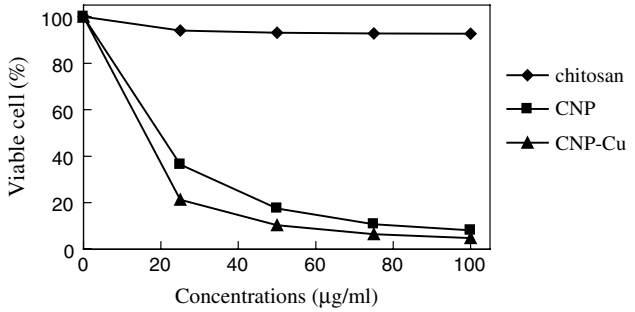

Figure 1. Cytotoxic activities of the chitosan, chitosan nanoparticles (mean particle size = 40 nm), and copper-loaded chitosan nanoparticles with various concentrations against BEL7402 cell line. CNP = chitosan nanoparticles; CNP-Cu = copper-loaded chitosan nanoparticles. Each value represents the mean of triplicate measurements and varied from the mean by not more than 10%.

BEL7402, BGC823, and Colo320 tumor cells, while having little effect on the growth of L-02 human normal liver cells. Figure 1 shows that these chitosan nanoparticles elicited dose-dependent inhibitory effects on the proliferation of the BEL7402 cell line. As shown in Figure 1, copper-loaded chitosan nanoparticles were highly tumor-suppressive. The IC_{50} values for chitosan nanoparticles and copper-loaded chitosan nanoparticles were, respectively, 15 and 6 μg/mL, much higher than values reported for other chitosan derivatives (IC_{50} 20–2500 μg/mL).[4,5] Zeta potential, that is, surface charge can greatly influence the particle stability in suspension through the electrostatic repulsion between the particles. The greater the zeta potential, the more likely the suspension is to be stable because the charged particles repel one another and thus overcome the natural tendency to aggregate.[11] Zeta potential of nanoparticles is also an important factor to determine their interaction in vivo with the tumor cell membrane, which is usually negatively charged.[12] The tumor cell uptake of nanoparticles can be viewed as a two-step process: first a binding step on the cell membrane and second the internalization step. Electrostatic interactions govern the adsorption of the nanoparticles onto the cell membrane.[13] The greater the zeta potential of nanoparticles, the stronger the interactions with tumor cell membrane, and leads to higher cytotoxicity. As a kind of cationic polymers, the surface charge of chitosan derivatives is the major factor affecting its cytotoxic activity due to the electrostatic ionic interaction between the negatively charged groups of the tumor cells and the positively charged amino groups of the chitosan.[5] The zeta potential of chitosan nanoparticles is 51 mV, while that of copper(II)-loaded chitosan nanoparticles increases to 96 mV due to the cupric (2^+) ion adsorbed on the surface of chitosan nanoparticles.[9] Therefore, the high surface charge of chitosan nanoparticles and copper(II)-loaded chitosan nanoparticles prepared in our studies is responsible for their higher cytotoxic activity. The cupric ion [Cu(II)] had been involved in curing of cancer, and copper(II) complexes synthesized by copper sulfate or nitrate were also reported to exhibit antitumor activity.[14,15] Copper(II)-loaded chitosan nanoparticles had been reported to show superior affinity with negativly charged cell membranes due to the chelation theory and higher surface charge density compared to chitosan nanoparticles without copper ion,[9] which can also explain the higher cytotoxic activity of copper(II)-loaded nanoparticles combined with the controlled release of cupric ion dissociated in chitosan nanoparticles. Necrotic morphological features of cells treated with chitosan nanoparticles such as loss of membrane integrity, disruption of the cytoplasm, and appearance of remnants of swollen organelles were also confirmed by a transmission electron microscopy picture (data not shown).

Since particle size has a crucial impact on the in vivo fate of a particulate drug delivery system,[16] control over the particle size is of great importance for drug carriers. According to Figure 2, the cytotoxic activity of the chitosan nanoparticles increased with decreasing particle size, the IC_{50} values for different particle size as 40, 70, and 100 nm of chitosan nanoparticles were, respectively, 14.98, 16.54, and 23.84 μg/mL, indicating that the smaller chitosan nanoparticles exhibit higher antitumor activity due to the increased accumulation targeting at tumor cells. Particle size of nanoparticles had been reported to play an important role in their antitumor activity and in vivo distribution. Small lipid nanospheres showed higher accumulation at tumor sites and prolonged in vivo half-life due to their avoidable capture by the reticuloendothelial system.[17,18] Small copolymer nanoparticles could stay in circulation for a long period resulting in better drug availability and enhance the in vitro release of the loaded drug.[19] On the other side, nanoparticles could also diminish the adverse effects of a drug associated with its use under conventional pharmaceutical dosage forms and improve bioavailability after administration.[20] Size-dependent cytotoxicity effect of realgar nanoparticles on the endothelial cell line suggested the less toxicity of smaller nanoparticles.[21]

For copper-loaded chitosan nanoparticles at a concentration of only 50 μg/mL, tumor cell proliferation was inhibited from 67% to 90% in the three cancer cell lines tested in the order Calo320 < BGC823 < BEL7402 (Fig. 3). Of these cell lines, the IC_{50} values were lowest in the BEL7402 cell line (Table 1). Moreover, chitosan nanoparticles showed high cytotoxic activity toward tumor

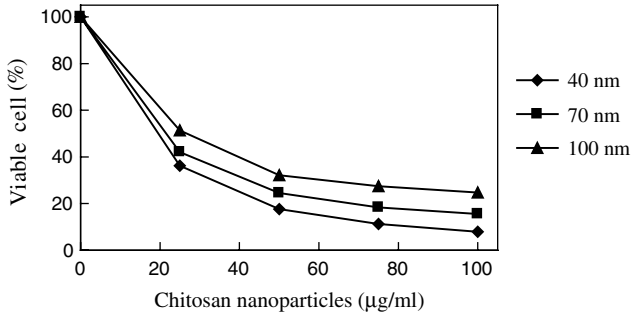

Figure 2. Cytotoxic activities of the chitosan nanoparticles with different mean particle size against BEL7402 cell line. Each value represents the mean of triplicate measurements and varied from the mean by not more than 10%.

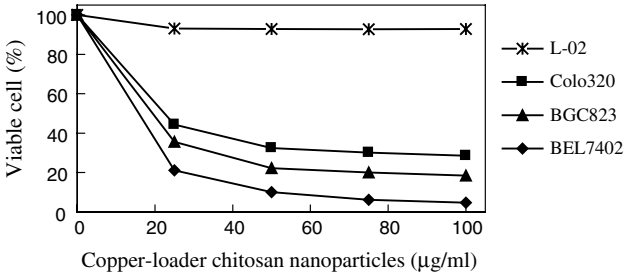

Figure 3. Cytotoxic activities of copper-loaded chitosan nanoparticles against different cell lines L-02, Colo320, BGC823, and BEL7402. Each value represents the mean of triplicate measurements and varied from the mean by not more than 10%.

Table 1. Cytotoxic activity of chitosan, chitosan nanoparticles (mean particle size = 40 nm), and copper-loaded chitosan nanoparticles against different cell lines

Panel of cell lines	Cell line	Cytotoxicity (IC_{50}, μg/mL)[a]		
		Chitosan	CNP	CNP-Cu
Liver	L-02	na	na	na
Colon cancer	Calo320	1200 (±27)	28 (±3)	14 (±2)
Gastric cancer	BGC823	1200 (±35)	21 (±2)	8 (±1)
Liver cancer	BEL7402	1200 (±22)	15 (±2)	6 (±1)

Values are means of three experiments, standard deviation is given in parentheses (na = not active).

cells, while low toxicity against normal human liver cells (L-02). Therefore, this study supports the application of chitosan nanoparticles in further preclinical and clinical studies involving a broad spectrum of malignant tumors due to its high cytotoxicity toward tumor cells and fewer side effects for nice biocompatibility than most anticancer chemotherapeutic agents. The details of the action mechanisms of chitosan nanoparticles in vitro and in vivo are now under investigation, especially to clarify the interaction mode between nanoparticles and tumor cells.

References and notes

1. Qin, C.; Du, Y.; Xiao, L.; Li, Z.; Gao, X. *Int. J. Biol. Macromol.* **2002**, *31*, 111.
2. Sudarshan, N. R.; Hoover, D. G.; Knorr, D. *Food Biotechnol.* **1992**, *6*, 257.
3. Tsai, G. J.; Su, W.-H. *J. Food Prot.* **1999**, *62*, 239.
4. Carreno-Gomez, B.; Duncan, R. *Int. J. Pharm.* **1997**, *148*, 231.
5. Lee, J.-K.; Lim, H.-S.; Kim, J.-H. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 2949.
6. Janes, K. A.; Fresneau, M. P.; Marazuela, A.; Fabra, A.; Alonso, M. J. *J. Control. Release* **2001**, *73*, 255.
7. De Campos, A. M.; Sanchez, A.; Alonso, M. J. *Appl. Cyclosorin A, Int. J. Pharm.* **2001**, *224*, 159.
8. Xu, Y.; Du, Y. *Int. J. Pharm.* **2003**, *250*, 215.
9. Qi, L.; Xu, Z.; Jiang, X.; Hu, C.; Zou, X. *Carbohydr. Res.* **2004**, *339*, 2693.
10. Qi, L.; Xu, Z. *Colloid Surf. A* **2004**, *251*, 183.
11. Mu, L.; Feng, S. S. *J. Control. Release* **2001**, *76*, 239.
12. Dong, Y.; Feng, S.-S. *Biomaterials* **2004**, *25*, 2843.
13. Wilhelm, C.; Billotey, C.; Roger, J.; Pons, J. N.; Bacri, J.-C.; Gazeau, F. *Biomaterials* **2003**, *24*, 1001.
14. Majumder, S.; Panda, G. S.; Choudhuri, S. K. *Eur. J. Med. Chem.* **2003**, *38*, 893.
15. Kong, D.; Qin, C.; Meng, L.; Xie, Y. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 1087.
16. Moghimi, S. M.; Hunter, A. C.; Murray, J. C. *Pharmacol. Rev.* **2001**, *53*, 283.
17. Gabizon, A.; Price, D. C.; Huberty, J. B.; Resalier, R. S.; Papahadjopoulos, D. *Cancer Res.* **1990**, *50*, 6731.
18. Takenaga, M. *Adv. Drug Deliver. Rev.* **1996**, *20*, 209.
19. Zhang, L.; Hu, Y.; Jianga, X.; Yang, C.; Lu, W.; Yang, Y. H. *J. Control. Release* **2004**, *96*, 135.
20. Molpeceres, J.; Guzman, M.; Aberturas, M. R. *J. Pharm. Sci.* **1996**, *85*, 206.
21. Deng, Y.; Xu, H.; Huang, K.; Yang, X.; Xie, C.; Wu, J. *Pharmacol. Res.* **2001**, *44*, 513.