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In vivo antitumor activity of chitosan nanoparticles

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Abstract—Chitosan nanoparticles have been synthesized as potential anticancer agents, and evaluated, in vitro, against various cancer cell lines. In this study, in vivo antitumor activity of chitosan nanoparticles against Sarcoma-180 and mouse hepatoma H22 was investigated. Chitosan nanoparticles showed significant antitumor activity in vivo. The doses and particle size made a great effect on their efficacy.

Chitosan nanoparticles have been synthesized as drug carriers as reported in previous studies.[1–3] The unique character of nanoparticles for their small size and quantum size effect could make chitosan nanoparticles exhibit biological activities.[4] Chitosan nanoparticles with little particle size and enhanced zeta potential had been prepared and characterized in our previous reports,[4,5] and their in vitro cytotoxic effects against various human tumor cell lines have been also studied. It showed that chitosan nanoparticles with little particle size and positive surface charge could exhibit higher antitumor activity than others chitosan derivatives, and the physiochemical properties of nanoparticles such as particle size and zeta potential could make a significant effect on their efficacy.[6] The antitumor mechanism of chitosan nanoparticles was related to its membrane-disrupting and apoptosis-inducing activities.[7] In this study, the in vivo antitumor activity of chitosan nanoparticles was investigated using Sarcoma-180 (S-180) and mouse hepatoma H22 (H22) bearing mice, and the effects of administration doses, administration routes and particle size are discussed.

Chitosan nanoparticles with mean particle size ranging from 40 to 100 nm and positive surface charge about 50 mV were prepared and characterized in our previous reports.[4,5] 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.[4]

We evaluated the in vivo antitumor activity of chitosan nanoparticles with different particle size, against the mouse tumor model: S-180 tumor and H22 with chitosan and cisplatin (cDDP) used as reference drugs, 0.9% saline solution was used as blank control. Drugs were administered from the fifth day after establishing the mouse model, when the volume of subcutaneous tumor in mice grew about 3 mm^3. Chitosan nanoparticles with different doses and different particle size were administered once daily by intravenous injection (iv), intraperitoneal injection (ip) and oral administration (po) respectively for consecutive 7 days.

The results presented here showed that chitosan nanoparticles exhibited very impressive antitumor efficacy in vivo against S-180 and H22, higher than chitosan group (Tables 1 and 2). Chitosan nanoparticles elicited dose-dependent tumor-weight inhibitions (TWI), the efficacy at dose of 2.5 mg/kg achieved 53% and 59% against S-180 and H22, respectively, by oral administration. At the same dose of 0.5 mg/kg by oral administration, chitosan nanoparticles showed much higher efficacy than chitosan group. The TWI against S-180 and H22 reached 43% and 52%, respectively, while that of chitosan group was just about 30%. cDDP as positive control drug exhibited higher antitumor efficacy than chitosan nanoparticles. However, cisplatin also showed

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Table 1. Assessment of antitumor efficacy of chitosan nanoparticles with different particle size against mouse tumor Sarcoma-180 using different administration routes

Groups	Dose (mg/kg)	AR[a]	Lethal[b] toxicity	BWC[c] (%)	TW[d]	TWI[e] (%)
Saline	0.5	iv	6/12	12.10	2.70 ± 0.36	—
CNPa	2.5	po	0/10	10.41	1.28 ± 0.38	53
CNPa	1	po	0/10	10.15	1.42 ± 0.29	47
CNPa	0.5	po	0/10	9.60	1.54 ± 0.35	43
CNPa	0.5	ip	0/10	8.36	1.62 ± 0.33	40
CNPa	0.5	iv	0/10	11.23	1.37 ± 0.41	49
CNPb	0.5	iv	0/10	10.52	1.73 ± 0.39	36
CNPc	0.5	iv	0/10	10.74	1.81 ± 0.46	33
Chitosan	0.5	po	0/10	9.57	1.85 ± 0.42	31
cDDP	0.5	iv	4/10	−13.57	1.18 ± 0.42	56

CNPa, CNPb, CNPc mean chitosan nanoparticles with particle size as 40, 70 and 100 nm, respectively, the same volume of 0.9% saline was used as control group.

[a] Administration routes, iv means intravenous injection; po means oral administration; ip means intraperitoneal injection.

[b] Number of dead mice/total number of mice.

[c] Percentage of mouse body-weight change (BWC) after drug treatment: BWC% = (mean BW final day/BW first day × 100) − 100; '—' means body-weight decrease.

[d] Average tumor-weight after drug treatment.

[e] Percentage of tumor-weight inhibition (TWI) versus control mice.

Table 2. Assessment of antitumor efficacy of chitosan nanoparticles with different particle size against mouse hepatoma H22 using different administration routes

Groups	Dose (mg/kg)	AR[a]	Lethal[b] toxicity	BWC[c] (%)	TW[d]	TWI[e] (%)
Saline	0.5	iv	5/12	14.23	2.98 ± 0.32	—
CNPa	2.5	po	0/10	12.41	1.22 ± 0.28	59
CNPa	1	po	0/10	11.15	1.34 ± 0.21	55
CNPa	0.5	po	0/10	12.60	1.43 ± 0.39	52
CNPa	0.5	ip	0/10	10.36	1.46 ± 0.43	51
CNPa	0.5	iv	0/10	12.23	1.37 ± 0.47	54
CNPb	0.5	iv	0/10	11.52	1.72 ± 0.49	42
CNPc	0.5	iv	0/10	10.74	1.85 ± 0.36	38
Chitosan	0.5	po	0/10	10.57	2.01 ± 0.32	32
cDDP	0.5	iv	3/10	−11.57	1.16 ± 0.41	61

The meaning of abbreviations was prescribed in Table 1.

great side effects compared with chitosan and nanoparticles. Chitosan nanoparticles resulted in only a small decrease in body weight of the mice relative to the saline control, which indicated few side effects. On the contrary, cDDP led to a significant lose in body weight of mice. In saline control group, six and five of 12 mice, separately in S-180 and H22 mouse model, died due to the transfer of tumor at the seventh day, cDDP also had a lethal toxicity as 4/12 and 3/12, respectively, while in chitosan and nanoparticles groups, no lethal toxicity occurred for the inhibition of tumor growth. The histopathological slices from the liver and kidney tissues were also examined by microscope (data not shown here). It didn't show the pathological changes due to the nanoparticles administration, which also indicated few side effects of chitosan nanoparticles.

Antitumor efficacy of chitosan nanoparticles administered by different routes was studied. For S-180 bearing mouse model, at the same dose, the efficacy of chitosan nanoparticles (40 nm) by oral administration (po), intraperitoneal injection (ip) and intravenous injection (iv) was 43%, 40% and 49%, respectively (Table 1). As shown in Figure 1, chitosan nanoparticles by different administration routes could inhibit the growth of

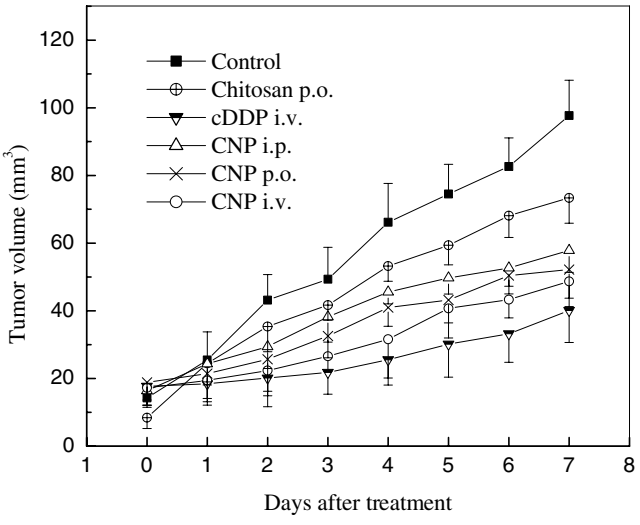

Figure 1. Effects of administration routes on antitumor efficacy of chitosan nanoparticles (CNP) against Sarcoma-180 subcutaneous tumor formation and growth in ICR mice. Changes in tumor volumes were measured every day. Each value represents mean ± SD of 10 animals. iv means intravenous injection; po means oral administration; ip means intraperitoneal injection. Chitosan and cisplatin (cDDP) were used as reference drugs.

S-180 tumor gradually and more effectively than chitosan group, and the tumor volume inhibition for intravenous injection (iv) group showed slightly more notable compared with oral administration (po) and intraperitoneal injection (ip) groups. For H22 bearing mouse model, TWI of chitosan nanoparticles by three administration routes achieved 52%, 51% and 54% separately. Results showed that chitosan nanoparticles could exhibit effective antitumor activities by different administration routes.

The oral route for colloidal drug carriers systems remains the most convenient and popular way of administration.[8] However, many anticancer drugs by oral administration are not bioavailable and adsorbable/interactive in the gastrointestinal tract, because the drugs could be eliminated from the first-pass extraction by the cytochrome P450-dependent metabolic processes and the overexpression of plasma membrane transporter P-glycoprotein (P-gp) in the physiological systems involved (intestine, liver, etc).[9] Application of nanoparticles with small size enough to improve the adhesion and absorption to the intestinal cells and to escape from the recognition of P-gp may provide better solutions for oral administration (po) of anticancer drugs. It is currently accepted that nanoparticles are taken up by the M-cells of Peyer's patches and the isolated follicles of the gut-associated lymphoid tissue and also via the enterocytes.[10] Recently, positively charged colloidal particles could increase the electrostatic interaction between the particles and the negatively charged mucin on the mucosal surface, thus improving their bioavailability and reducing their side effects.[11] In this study, chitosan nanoparticles, prepared and characterized previously, exhibited positive charge and little particle size, which is responsible for their in vivo efficacy.

Conventional colloidal carriers are rapidly removed from the bloodstream by the reticuloendothelial system (RES), which is a part of the mononuclear system (MPS) after intravenous (iv) administration.[12] Nanoparticulate systems have been used to improve the blood circulating time and tumor targeting efficacy of vincristine,[13] because the tumor vascular permeability allows the penetration of particles up to 400 nm in diameter.[14] Therefore, the antitumor efficacy of chitosan nanoparticles administered by intravenous injection (iv) is probably attributed to their little particle size.

Particle size had been proved as an important feature related to obtaining optimal in vitro efficacy of chitosan nanoparticles. Particles size also had a crucial impact on the in vivo fate of a particulate drug delivery system.[15] Decreasing particles size could increase the surface-to-volume ratio and specific surface area, which could increase the dissolution and thus increase bioavailability of poorly water soluble molecules.[16] The smaller size particles seem to have efficient interfacial interaction with the cell membrane compared to larger size particles due to the endocytosis of small size particles. Small size particles could improve efficacy of the particle-based oral drug delivery systems.[17] The use of particle size reduction to increase the oral bioavailability of drugs has been obtained.[18]

Nanoparticles could prolong the blood half-life of drugs and increase the efficacy by intravenous injection (iv).[19] If particles between 30 and 100 nm are intravenously applied, the liver eliminates the larger particles faster from the bloodstream compared to the smaller particles. Thus, the larger the particles are, the shorter is their plasma half-life-period.[20] In this study, with the increase of particle size, the efficacy of chitosan nanoparticles administrated by intravenous injection (iv) decreased significantly. The TWI of chitosan nanoparticles (40 nm) against S-180 and H22 was 49% and 54%, respectively.

In summary, chitosan nanoparticles exhibited impressive antitumor activity in S-180 and H22 bearing mouse. There is some difference among the efficacy for three administration routes, intravenous injection (iv) showed the higher TWI. Particle size also makes a great effect on their antitumor efficacy. Smaller the particle size, higher antitumor activity of chitosan nanoparticles by intravenous injection. The unique character with positive charge and little particle size of chitosan nanoparticles is responsible for their in vivo efficacy.

References and notes

1. Janes, K. A.; Fresneau, M. P.; Marazuela, A.; Fabra, A.; Alonso, M. J. *J. Control Rel.* **2001**, *73*, 255.
2. De Campos, A. M.; Sanchez, A.; Alonso, M. J. *Int. J. Pharm.* **2001**, *224*, 159.
3. Xu, Y.; Du, Y. *Int. J. Pharm.* **2003**, *250*, 215.
4. Qi, L.; Xu, Z.; Jiang, X.; Hu, C.; Zou, X. *Carbohydr. Res.* **2004**, *339*, 2693.
5. Qi, L.; Xu, Z. *Colloids Surf., A* **2004**, *251*, 183.
6. Qi, L.; Xu, Z.; Jiang, X.; Li, Y.; Wang, M. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 1397.
7. Qi, L.; Xu, Z.; Li, Y.; Jiang, X.; Han, X. *World J. Gastroentero.* **2005**, *11*, 5136.
8. Tobio, M.; Sanchez, A.; Vila, A.; Soriano, I.; Evora, C.; Vila-Jato, J. L.; Alonso, M. J. *Colloid Surf., B* **2000**, *18*, 315.
9. Feng, S.; Chien, S. *Chem. Eng. Sci.* **2003**, *58*, 4087.
10. Florence, A. T. *Pharm. Res.* **1997**, *14*, 259.
11. El-Shabouri, M. H. *Int. J. Pharm.* **2002**, *249*, 101.
12. Marianne, R.; Dagmar, F.; Thomas, K. *Eur. J. Pharm. Biopharm.* **1998**, *46*, 255.
13. Wang, J.; Kozo, T.; Tsuneji, N.; Yoshie, M. *Int. J. Pharm.* **2003**, *251*, 13.
14. Fan, F.; Marc, D.; Dai, F.; Michael, L.; David, A. B.; Torchilin, V. P.; Jain, R. K. *Cancer Res.* **1995**, *55*, 3752.
15. Moghimi, S. M.; Hunter, A. C.; Murray, J. C. *Pharmacol. Rev.* **2001**, *53*, 283.
16. Kondo, N.; Iwao, T.; Kikuchi, M.; Shu, H.; Yamanouchi, K.; Yokoyama, K.; Ohyama, K.; Ogyu, S. *Biol. Pharm. Bull.* **1993**, *16*, 796.
17. Kreuter, J. *Adv. Drug Deliv. Rev.* **1991**, *7*, 71.
18. Gary, G. L.; Kenneth, C. C. *Int. J. Pharm.* **1995**, *125*, 91.
19. Williams, J.; Lansdown, R.; Sweitzer, R.; Romanowski, M.; LaBell, R.; Ramaswami, R.; Unger, E. *J. Control Rel.* **2003**, *91*, 167.
20. Chouly, C.; Pouliquen, D.; Lucet, I.; Jeune, J. J.; Jallet, P. *J. Microencapsulation* **1996**, *13*, 245.