Intratumoral delivery of paclitaxel-loaded poly(lactic-*co*-glycolic acid) microspheres for Hep-2 laryngeal squamous cell carcinoma xenografts

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The introduction of induction chemotherapy provides an expectation of laryngeal function preservation without reduction in survival for patients with advanced laryngeal squamous cell carcinoma. The antitumor activity of conventional intravenous chemotherapy, however, is limited by systemic toxicity. The polymeric drug system delivered locally provides a novel modality of increasing therapeutic concentrations of drug for a prolonged period while decreasing systemic levels. In the current study, paclitaxel-loaded sustained-release microspheres were developed using poly(lactic-*co*-glycolic acid) as a drug carrier. Intratumoral administration of paclitaxel in the formulation of polymer showed enhanced efficacy against laryngeal squamous cell carcinoma in nude mice compared with conventional paclitaxel injection via the intratumoral or intraperitoneal route. No significant toxic reactions were observed in the experiment. Immunohistochemical findings indicated that paclitaxel exhibited antiangiogenic activity by inhibiting the expression of basic fibroblast growth factor and vascular endothelial growth factor within the tumor. Moreover, this effect could be better exploited via localized delivery of polymeric paclitaxel. In conclusion, direct administration of polymeric drug system at the tumor sites proved to be promising for the treatment of laryngeal carcinoma. *Anti-Cancer Drugs* **18:459–466 © 2007 Lippincott Williams & Wilkins.**

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Introduction

For a long time, laryngectomy and radiotherapy have been the two major strategies for the treatment of laryngeal carcinoma. Since Laccourreye's group [1,2] pioneered the concept and use of induction chemotherapy in patients with invasive squamous cell carcinoma of the true vocal cord in the early 1980s, the role of induction chemotherapy in the preservation of laryngeal function without a detrimental effect on survival in patients with advanced laryngeal carcinoma has been demonstrated by a large number of clinical trials [3–6]. The modest efficacy of about 30% rate of complete clinical response [7,8] and high incidence of side effects, however, commonly observed with systemic chemotherapy, clearly deduced the need for an alternate formulation and administration method. The delivery of chemotherapeutic agents to the tumor site using a polymeric system makes it possible to achieve high local concentration of drugs, while minimizing systemic toxicity [9].

Paclitaxel, a potent anticancer agent, has demonstrated significant activity in clinical trials against a variety of tumors [10]. It has been used in the treatment regimen for organ preservation in patients with locally advanced head and neck squamous cell carcinoma [11–13]. Paclitaxel has a unique mechanism of action to promote the polymerization of tubulin resulting in the formation of highly stable microtubules, thus preventing cell division [14]. In addition to the antiproliferative effects, it has been shown to block cell invasion and metastases by its combined inhibitory activity on protease secretion, cell attachment/detachment and mobility [15]. Moreover, paclitaxel was found to have broad inhibitory effects on angiogenesis, including proliferation, motility and cord formation of endothelial cells [16,17], and vascular endothelial growth factor (VEGF)-induced neovascularization [18].

With multiple pharmacological effects, paclitaxel seems to be of tremendous potential in the treatment of cancer. More effective clinical application of the drug is, however, confined by the poor water-solubility. Cremophor EL (BASF Chemicals, Ludwigshafen, Germany), which can cause serious hypersensitivity reactions, is used as adjuvant in current commercial injections [19]. Localized delivery of paclitaxel by new formulations such as microspheres [20], microparticles [21], surgical pastes [22] and implants [23] has been attempted to increase the antitumor efficacy of the drug, while at the same time to also eliminate the toxicity of Cremophor EL.

In the present study, paclitaxel was incorporated into poly(lactic-*co*-glycolic acid) (PLGA), which has been used as a polymeric vehicle for different chemotherapeutic agents because of its biodegradation and excellent biocompatibility [24–27]. Although attempts have been made to prepare paclitaxel-loaded PLGA microspheres [28–30], the in-vivo antitumor activity has not been investigated. The purpose of this study was to assess the efficacy of the developed paclitaxel-loaded PLGA microspheres delivered locally in a Hep-2 murine laryngeal squamous cell carcinoma model, and to examine the counts of microvessel density (MVD) and the expression of basic fibroblast growth factor (bFGF) and VEGF in xenografts to evaluate the antiangiogenic activity of the polymer via localized delivery.

Materials and methods

Materials

Paclitaxel and paclitaxel injection were obtained from Sichuan Taiji Pharmaceutical (Sichuan, China). PLGA (L/G = 75:25, mol. wt. of 15 000) was purchased from Sichuan Zhuoxin Biomaterial (Sichuan, China). Polyvinyl alcohol was purchased from Sigma (St Louis, Missouri, USA). Dichloromethane (DCM) and methanol [high-pressure liquid chromatography (HPLC) grade] were bought from Tedia (Fairfield, Ohio, USA). All other chemicals used were of analytical grade. The Hep-2 cell line was provided by the Cell Bank of the Chinese Institute of Biochemistry and Cell Biology. Female athymic nude BALB/c mice were obtained from Shanghai Cancer Institute.

Preparation of paclitaxel-loaded poly(lactic-*co*-glycolic acid) microspheres

The paclitaxel-loaded PLGA microspheres were prepared by the modified solvent evaporation method [28]. Paclitaxel was dissolved in DCM containing 2% PLGA using a probe sonicator. The resulting organic phase was then slowly poured into an aqueous phase containing 2% (w/v) polyvinyl alcohol while being stirred at 800 ± 10 r.p.m. at room temperature to evaporate DCM. The microspheres were collected by filtration through a 0.45-μm membrane filter, washed 2 times with distilled water and dried at room temperature. The produced microspheres were radiosterilized at 25 kGy.

Drug loading and encapsulation efficiency

To determine the paclitaxel content in the microspheres, 10 mg of microspheres was dissolved in 0.5 ml of DCM using a probe sonicator and mixed with 2.5 ml of methanol. The mixture was vortexed vigorously for 10 min and centrifuged at 8000 r.p.m. for 10 min. The supernatant was then taken for analysis of paclitaxel concentration by HPLC. Drug loading is defined as the ratio of amount of drug in microspheres to that of microspheres. Encapsulation efficiency is defined as the ratio of amount of drug encapsulated to that of the drug used in microsphere preparation.

High-pressure liquid chromatography assay for determination of paclitaxel content

The samples were analyzed using a LC-10Atvp HPLC system (Shimadzu, Columbia, Maryland, USA). A mobile phase of methanol and water (80 : 20, (v/v)) was passed through a Diamonsil C18 column (250 × 4.6 mm, pore size 5 μm, Dikma; Beijing, China) at a flow rate of 1.0 ml/min. The column temperature was maintained at 30°C. Paclitaxel was detected at 227 nm. The concentration of paclitaxel was determined from the peak area correlated with the standard curve. The standard curve was linear over the range of standard solutions with a correlation coefficient of $R^2 = 0.9999$.

Particle size determination and surface morphology

Particle size and size distribution were measured by the laser light scattering technique (AccuSizer 780; Particle Sizing Systems, Santa Barbara, California, USA). The shape and surface morphology of the microspheres were examined with a scanning electron microscope (SEM; Hitachi S-520, Tokyo, Japan). The sample was placed on a graphite surface and coated with gold using an ion sputter. The microspheres were viewed at an accelerating voltage of 20 kV.

In-vitro release

Twenty-five milligrams of microspheres were suspended in 2 ml of phosphate-buffered saline (pH 7.4) containing 3% polyethylene glycol 6000 in screw-capped tubes. After being vortexed for 1 min, tubes were incubated at 37°C and shaken horizontally at (75 ± 5) r.p.m. At given time intervals (1, 5, 10, 15, 20, 25 and 30 days), three tubes were taken out of the shaker and centrifuged at 8000 r.p.m. for 10 min. The supernatant was taken for analysis of paclitaxel amount by HPLC. The precipitated microspheres were resuspended in 2 ml of fresh phosphate-buffered saline containing 3% polyethylene glycol 6000 and shaken at 37°C.

Tumor growth *in vivo*

Hep-2 laryngeal squamous cell carcinoma was maintained by serial passage in female BALB/c nude mice. Mice weighing 18–20 g were inoculated subcutaneously with 1.5 mm^3 of tumor tissue. When the mean tumor diameter reached about 8 mm, the animals were randomly assigned to one of the six treatment groups consisting of six mice each: (1) intraperitoneal (i.p.) injection of paclitaxel injection, 15 mg/kg, twice a week for 3 weeks; (2) a single intratumoral (i.t.) injection of saline; (3) a single i.t. injection of blank micropheres; (4) a single i.t. injection of paclitaxel injection, 15 mg/kg; (5) a single i.t. injection of paclitaxel-loaded PLGA microspheres, 15 mg/kg; (6) a single i.t. injection of paclitaxel-loaded PLGA microspheres, 25 mg/kg.

Paclitaxel-loaded microspheres in this study was suspended in saline for direct i.t. injection. For each injection, the total volume was 150 µl. For the i.t. injection, one-half was injected in the center of the tumor and the other half was injected around the tumor in an attempt to perfuse the tumor tissue completely.

Tumor size and weight of the mice were measured twice a week until the animals were killed. Tumor measurements were made with calipers and volumes were calculated using the formula $V = (ab^2)/2$, where a is the longest diameter (mm) and b is the diameter orthogonal to a (mm). On day 21 after treatment, animals were killed. Tumors were excised and weighed before being fixed in 10% neutral-buffered formalin. The percent inhibition rate (IR) was evaluated as follows: IR = (1 – weight of treated tumor/weight of control tumor) × 100. The tumor volume tripling time was calculated from the linear regression analysis of log (tumor volume) versus log (time) profiles. All animal procedures were approved by the Institutional Animal Care and Use Committee of China.

Immunohistochemical staining

Immunohistochemical staining was performed on 4-µm formalin-fixed, paraffin-embedded tumor tissues. Sections were deparaffinized with xylene and rehydrated in graded alcohol. The sections for CD34 and VEGF were heated in a microwave oven for 10 min, in 0.01 mol/l citric acid buffer, pH 6.0. The sections for bFGF were immersed with 0.1% trypsin for 20 min at room temperature. Three-percent hydrogen peroxide was then applied to block endogenous peroxidase activity and nonspecific reaction was blocked with blocking solution. The sections were incubated overnight at room temperature with antihuman CD34 mouse polyclonal antibody (Dako; Glostrup, Denmark), antihuman VEGF mouse monoclonal antibody (Dako; Fouzhou, China) and antihuman bFGF rabbit polyclonal antibody (Maixin; RAB 0305). Immunostaining was performed with the Envision system (Dako). 3,3′-Diaminobenzidine was used as a chromogen. All sections were counterstained with hematoxylin. For negative control samples, Tris-buffered saline (pH 7.4) was applied to the sections replacing the primary antibody.

The results were evaluated by two pathologists who were blinded to the drug administration. MVD was assessed based on the criteria of Weidner *et al.* [31]. Any brown-stained endothelial cell or endothelial cell cluster that was clearly separate from the adjacent microvessels, tumor cells and other connective tissue elements was considered a single, countable microvessel. 'Hot spots' with the highest vascularity were selected from non-necrotic areas at low-power magnification (×100). Microvessels were counted within five fields randomly selected from these 'hot spots' at high-power magnification (×200). MVD was expressed as the number of stained microvessels per optical field.

The scoring method described by Pavelic *et al.* [32] was followed for the evaluation of VEGF and bFGF expression. VEGF and bFGF staining was considered positive when appropriate brown staining was seen in the cytoplasm tumor cells. Staining intensity was graded into four categories: 0 (none), 100 (weak), 200 (moderate) and 300 (strong). The scores were determined by multiplying the staining values by the percentage of positive stained cells.

Statistical analysis

Statistical analysis was carried out using the SPSS 11.5 program package (SPSS; Chicago, Illinois, USA). Experimental data were evaluated by one-way anlaysis of variance in conjunction with post-hoc Tukey's test. P values less than 0.05 were considered as statistically significant.

Results

Characterization of the microspheres

In the present study, paclitaxel was incorporated into the biodegradable polymer PLGA as a controlled release formulation of the anticancer agent. As examined by the scanning electron microscope (Hitachi S-520), the prepared microspheres were spherical in shape and had a smooth surface (Fig. 1). The mean particle size was 42.72 µm. The drug loading was 1.53% and the encapsulation efficiency was 97.29%.

In-vitro release

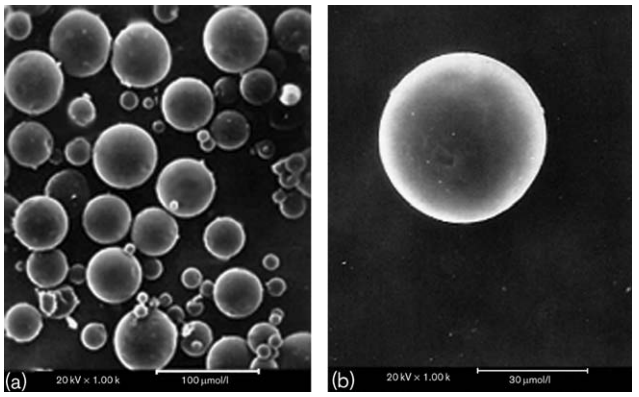
The in-vitro release profile is shown in Fig. 2. The paclitaxel-loaded PLGA microspheres released 6.83% paclitaxel in the first 24 h. This initial release was followed by a controlled release with a cumulative amount of 53.53% in a 30-day period.

In-vivo antitumor activity

The in-vivo antitumor effects of paclitaxel-loaded PLGA microspheres were investigated in a murine model of Hep-2 laryngeal squamous cell carcinoma. It was noted that all mice receiving i.p. administration of conventional paclitaxel injection huddled up for 5–10 min after administration, whereas no significant systemic toxic reactions were observed in other mice during the experimental period. Animal weights increased in all the groups over time without significant differences between groups ($P > 0.05$). The skins around the injection sites were intact without edema or ulceration.

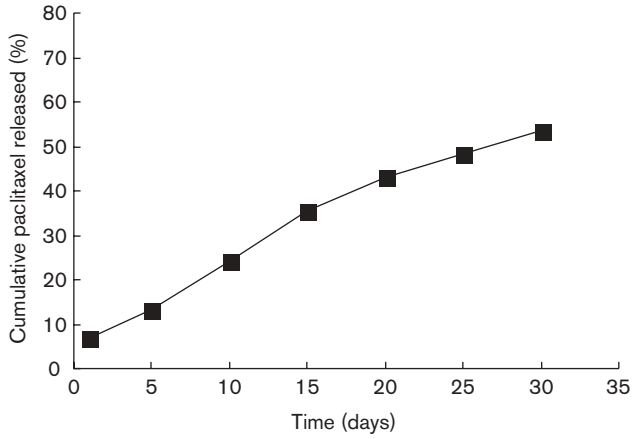
The effects of paclitaxel on tumor growth are shown in Fig. 3. The two control groups (i.t. saline and i.t. blank micropheres) showed a steady increase in tumor volume throughout the 21-day post-treatment period and no significant difference was found between them

Fig. 1



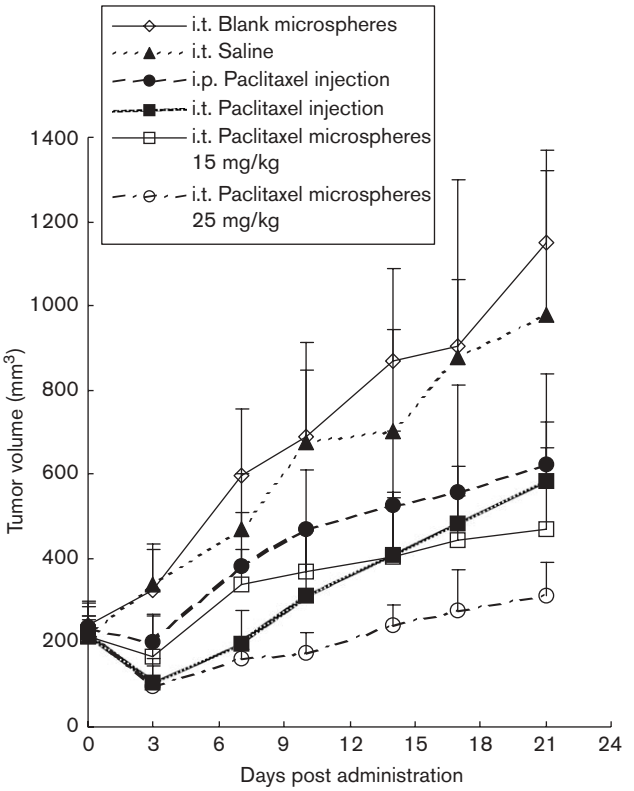
Scanning electron micrographs of paclitaxel-loaded microspheres. (a) microspheres exhibit round morphology with smooth surface (scale bar = 100 mm); (b) micrograph of microspheres at high power magnification (scale bar = 30 mm)

Fig. 2



In-vitro release of paclitaxel from microspheres.

Fig. 3



The change in tumor volume as a function of time after different treatments in Hep-2 laryngeal squamous cell carcinoma-bearing nude mice. The results are expressed as mean ± SD ($n = 6$).

Table 1 The mean tumor weight and inhibition rate for Hep-2 laryngeal squamous cell carcinoma xenografts treated with different schedules in nude mice ($n = 6$)

Group	Tumor weight (g)	IR (%)
i.t. Saline	0.69 ± 0.24	–
i.t. Blank microspheres	0.88 ± 0.17[a]	–
i.p. Paclitaxel injection 15 mg/kg	0.44 ± 0.17[b]	35.99
i.t. Paclitaxel injection 15 mg/kg	0.42 ± 0.19[b]	39.37
i.t. Paclitaxel microspheres 15 mg/kg	0.36 ± 0.16[c]	47.83
i.t. Paclitaxel microspheres 25 mg/kg	0.28 ± 0.10[d]	59.90

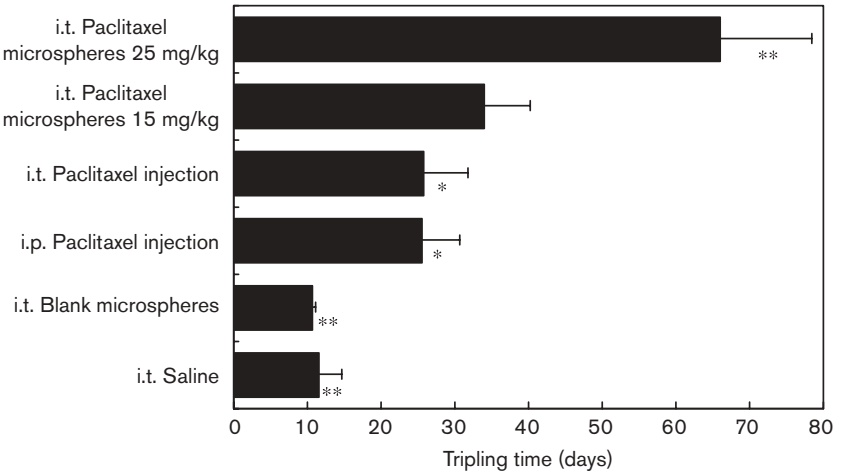
i.t., intratumoral; i.p., intraperitoneal.
[a] $P > 0.05$ versus saline control group.
[b] $P < 0.05$ versus saline control group.
[c] $P < 0.01$ versus saline control group.
[d] $P < 0.05$ versus i.p. and i.t. paclitaxel injection groups.

($P > 0.05$), indicating that the blank polymers had no effect on tumor growth. Twenty-one days after treatment, the tumor size of paclitaxel-treated groups was significantly reduced compared with the saline control group ($P < 0.01$). The i.t. administration of paclitaxel microspheres at 25 mg/kg produced a marked reduction in tumor growth in comparison with i.p. and i.t. administration of paclitaxel injection ($P < 0.01$).

The mean tumor weight and inhibition rate are shown in Table 1. The inhibition rate of the paclitaxel microspheres 15 mg/kg group was 47.83%, compared with 35.99 and 39.37% for the same dose of paclitaxel injection given via the i.t. and i.p. route, respectively. The treatment of paclitaxel microspheres at 25 mg/kg resulted in a significant reduction in tumor weight compared with the i.p. and i.t. administration of paclitaxel injection ($P < 0.05$), with the inhibition rate of 59.90%.

As shown in Fig. 4, the average tumor volume tripling time increased from 11.63 days in the saline control group to 34.07 days in the paclitaxel microspheres 15 mg/kg group and to 65.91 days in the paclitaxel microspheres 25 mg/kg group. Groups receiving paclitaxel microspheres

Fig. 4



Estimated tumor volume tripling time for Hep-2 laryngeal squamous cell carcinoma xenografts treated with different schedules in nude mice. The results are expressed as mean ± SD ($n=6$). *$P<0.05$ versus paclitaxel microspheres 15 mg/kg group. **$P<0.01$ versus paclitaxel microspheres 15 mg/kg group.

Table 2 MVD counts and the expression of bFGF and VEGF in Hep-2 laryngeal squamous cell carcinoma xenografts treated with different schedules in nude mice ($n=6$)

Group	MVD ($\bar{x} \pm s$)	bFGF scores ($\bar{x} \pm s$)	VEGF scores ($\bar{x} \pm s$)
i.t. Saline	9.50 ± 1.34	225.00 ± 26.83	230.00 ± 24.49
i.t. Blank microspheres	9.37 ± 1.28[a]	214.17 ± 45.98[a]	198.33 ± 39.20[a]
i.p. Paclitaxel injection 15 mg/kg	7.98 ± 1.46[b]	181.67 ± 35.02[b]	155.00 ± 19.75[b]
i.t. Paclitaxel injection 15 mg/kg	7.55 ± 1.57[b]	175.83 ± 38.78[b]	126.67 ± 15.06[b]
i.t. Paclitaxel microspheres 15 mg/kg	3.63 ± 0.76[c]	60.83 ± 24.17[c]	138.33 ± 31.25[b]
i.t. Paclitaxel microspheres 25 mg/kg	3.30 ± 0.98[c]	58.33 ± 24.01[c]	120.00 ± 40.50[b]

MVD, microvessel density; bFGF, basic fibroblast growth factor; VEGF, vascular endothelial growth factor; i.t., intratumoral; i.p., intraperitoneal.
[a]$P>0.05$ versus saline control group.
[b]$P<0.05$ versus saline control group.
[c]$P<0.01$ versus i.p. and i.t. paclitaxel injection groups.

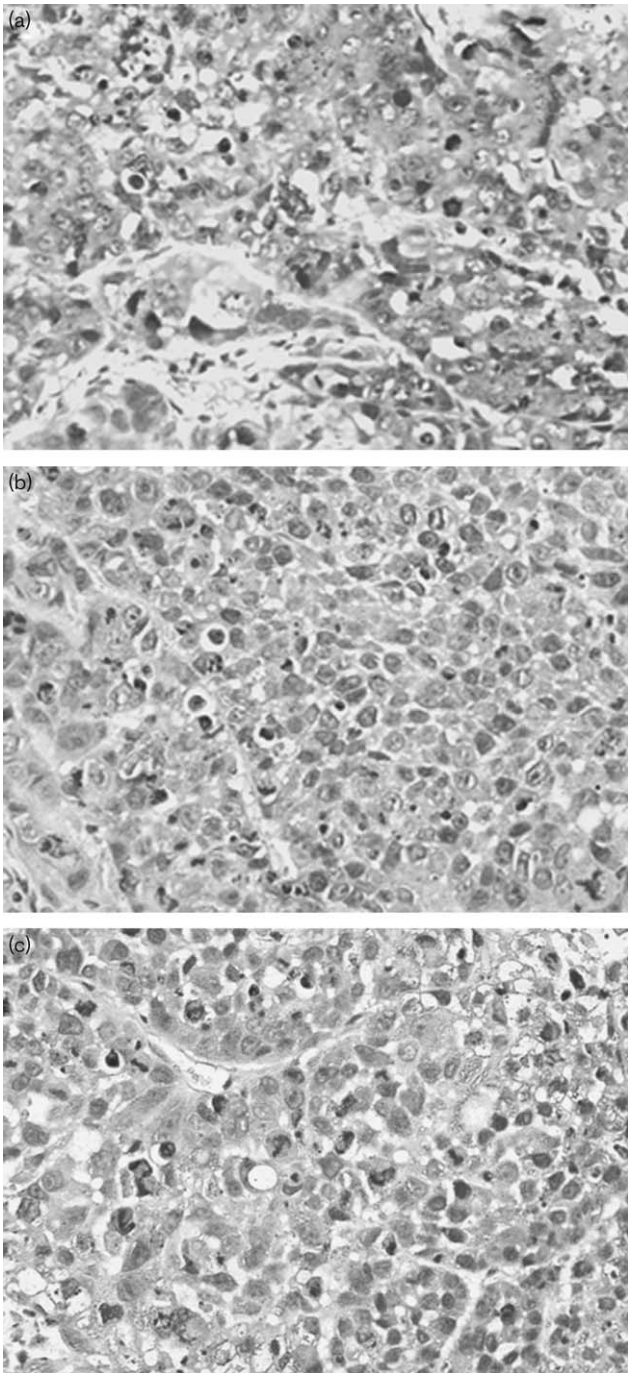
had significantly prolonged tumor volume tripling time when compared with conventional paclitaxel injection groups by i.t. or i.p. administration ($P < 0.05$) and paclitaxel microspheres 25 mg/kg produced the longest tumor volume tripling time. These findings suggested that localized delivery of paclitaxel microspheres was more effective than paclitaxel solution via the i.t. or i.p. route in inhibiting the growth of Hep-2 laryngeal squamous cell carcinoma in nude mice.

Microvessel density and expression of basic fibroblast growth factor and vascular endothelial growth factor

To evaluate the effects of paclitaxel on tumor-associated angiogenesis, tissue sections are stained with anti-CD34 antibody. MVD counts following different treatments are shown in Table 2. Xenografts treated with paclitaxel showed a significantly lower level of MVD than controls (saline and blank microspheres) ($P < 0.05$). The counts of MVD in tumors treated with paclitaxel microspheres were significantly decreased compared with those in conventional paclitaxel injection-treated tumors ($P < 0.01$).

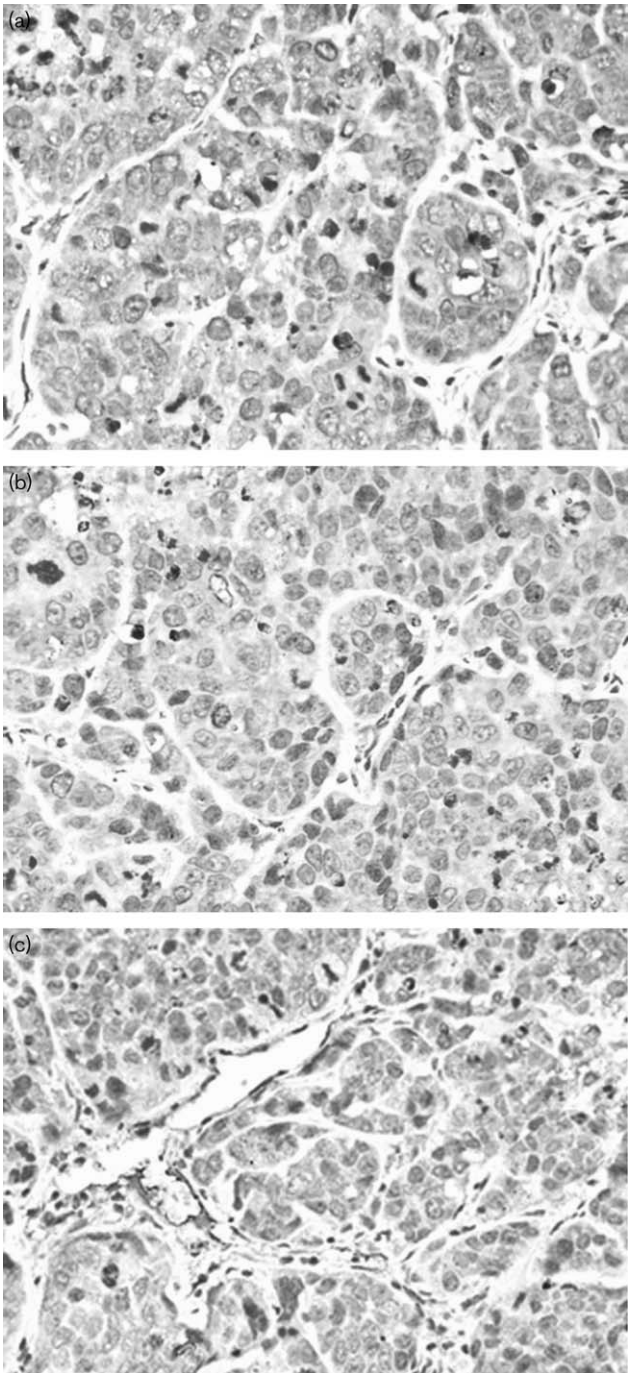
Positive immunostaining with bFGF and VEGF was predominantly observed in the cytoplasm of tumor cells. A strong staining of bFGF and VEGF was present in xenografts receiving saline (Figs 5a and 6a) and blank microspheres. As shown in Table 2, the expression of bFGF and VEGF was significantly decreased in each of the paclitaxel-treated tumors compared with the saline control ($P < 0.05$). The level of bFGF in tumors treated with paclitaxel microspheres was significantly lower than that in conventional paclitaxel injection-treated tumors ($P < 0.01$) (Fig. 5b and c). The immunoreactivity of VEGF in paclitaxel microspheres-treated tumors, however, was not significantly different from that in conventional paclitaxel injection-treated ones ($P > 0.05$) (Fig. 6b and c). These results indicated that paclitaxel inhibited the expression of angiogenic factors in tumor cells *in vivo*. In addition, the localized sustained release of paclitaxel from the polymer resulted in the

Fig. 5



Immunohistochemical staining of basic fibroblast growth factor (bFGF) in Hep-2 laryngeal squamous cell carcinoma xenografts treated with different schedules in nude mice. (a) Strong cytoplasmic staining of bFGF was seen in tumor receiving saline as a control; (b) decreased expression of bFGF was detected in tumor treated with conventional paclitaxel injection intraperitoneally compared with the saline control ($P<0.05$); (c) decreased expression of bFGF was noted in tumor treated with paclitaxel poly(lactic-*co*-glycolic acid) microspheres 25 mg/kg intratumorally compared with conventional paclitaxel injection-treated tumors ($P<0.01$), $\times$ 200.

Fig. 6



Immunohistochemical staining of vascular endothelial growth factor (VEGF) in Hep-2 laryngeal squamous cell carcinoma xenografts treated with different schedules in nude mice. (a) Strong cytoplasmic staining of VEGF was seen in tumor receiving saline as a control; (b) decreased expression of VEGF was detected in tumor treated with conventional paclitaxel injection intraperitoneally compared with the saline control ($P<0.05$); (c) decreased expression of VEGF was noted in tumor treated with paclitaxel poly(lactic-*co*-glycolic acid) microspheres 25 mg/kg intratumorally compared with the saline control ($P<0.05$), $\times$ 200.

strongest suppressive effects on tumor-induced bFGF expression.

Discussion

To investigate whether the polymeric delivery system may have potential implication in the treatment of laryngeal carcinoma, BALB/c nude mice bearing Hep-2 laryngeal squamous cell carcinoma were treated with paclitaxel PLGA microspheres. Paclitaxel administrated by a single i.t. injection in the formulation of polymer proved to be more effective than conventionally formulated paclitaxel administered by multiple i.p. injection, probably owing to the sustained high drug burden at the tumor site. Although paclitaxel release over time within the tumor nodules was not measured in the experiment, the in-vitro release data showed that paclitaxel was released slowly and sustainedly from the polymer during a long period of time, suggesting that the peak paclitaxel level in the paclitaxel microsphere-treated animals was lower than that in the group receiving i.t. injection of an equivalent dose of conventional paclitaxel injection. As seen in Fig. 3, intrarumoral administration of conventional paclitaxel injection delayed markedly tumor growth at the early stage, which may be due to the high drug concentration resulting from a rapid release within the first several days. The inhibitory effects, however, decreased markedly after 1 week. It has been demonstrated that paclitaxel is a cell-cycle-specific drug with the cells in late G_2 phase and mitosis being more sensitive than cells in the interphase. Thus, drug exposure duration is a crucial factor in paclitaxel cytotoxicity as more cells would enter the sensitive phase of the cell cycle [33]. More prolonged exposure to paclitaxel was shown to be the determinant of clinical response in breast cancer [34]. In addition, recent trials have tested paclitaxel administered by continuous infusions of up to 5 days [35] and 7 weeks [36] during radiation therapy for locally advanced head and neck squamous cell carcinoma. In view of the evidence that the antitumor effects of paclitaxel are dependent upon sustained modest concentrations of the drug rather than temporary high concentrations, i.t. administration of paclitaxel polymer seemed to be an optimal method of delivery.

In the preliminary study, we found that 60% of nontumor-bearing nude mice died when a single dose of 25 mg/kg conventional paclitaxel injection was administered i.p. (data not shown). In contrast, no systemic or local side effects were noted in the mice treated with paclitaxel microspheres at a dose of 25 mg/kg in the current study. The significantly improved antitumor efficacy of 25 mg/kg paclitaxel-loaded microspheres strengthen the validity of the polymeric delivery system administered directly into tumor sites, achieving safer and more effective outcomes.

Accumulating data indicate that paclitaxel has direct effects on endothelial cells [16,17]. Our results of improved antiangiogenic effects of paclitaxel microspheres, compared with free drug, are presumably related to the elevated sensitivity of vascular endothelial cells induced by continuous long-term exposure protocol, as confirmed by Bocci *et al.* [37]. In addition, the present study showed that reduced development of vascularization in the paclitaxel-treated groups may as well result from interfering with certain tumor cell functions relevant to angiogenesis, such as bFGF and VEGF production, and that paclitaxel microspheres exhibited stronger suppressive effects on bGFG expression. It is well-established that VEGF and bFGF are major stimulants of the vascularization of solid tumors [38,39]. Taken together, the results provide further evidence that localized delivery of paclitaxel in the formulation of polymer seems to capitalize and magnify the versatility of the drug, which makes it a more promising candidate for local drug therapy of cancer compared with the other currently available drugs [40].

Owing to the tiny volume, microspheres have an advantage of being injected into laryngreal tumors in the form of suspension under laryngoscopy avoiding open surgery and can be repeated if required. Localized delivery of anticancer agent-loaded microspheres is not only a valuable modality for induction chemotherapy but also promising for unresectable tumors, which involve important structures such as the carotid artery and skull base. In addition, it may provide palliation to recurrent cancer refractory to extensive surgery and radical radiotherapy.

In conclusion, localized delivery of paclitaxel-loaded PLGA microspheres into Hep-2 laryngeal squamous cell xenografts resulted in enhanced inhibitory activities against tumor growth and tumor neovascularizaion without obvious side effects. The results obtained from this study indicate that i.t. injection of paclitaxel in the formulation of microspheres is a promising modality for the treatment of laryngeal carcinoma.

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