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Original Article

Therapeutic efficacy of paclitaxel and carboplatin via arterial or venous perfusion in rabbits with VX-2 tongue cancer

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Abstract: Objective: This study aimed to investigate the therapeutic efficacy of paclitaxel in combination with carboplatin in different ways in rabbits with VX-2 tongue cancer. Methods: Rabbit VX-2 tongue cancer model was established and animals were then divided into 6 groups, in which animals received perfusion with paclitaxel liposome and carboplatin via the lingual artery, with free paclitaxel and carboplatin via the lingual artery, with 5% glucose via the lingual artery, with paclitaxel liposome and carboplatin via ear vein, with free paclitaxel and carboplatin via the ear vein and with 5% glucose via the ear vein independently. When the maximum diameter of cervical lymph nodes was larger than 5 mm, chemotherapy was initiated. Seven days later, flow cytometry and immunohistochemistry were performed to detect the apoptosis of VX-2 cells and P53 expression in the primary cancer and metastatic lymph nodes. Results: Targeted arterial perfusion with paclitaxel liposome in combination with carboplatin was more effective to induce the apoptosis of cancer cells in the primary cancer and metastatic lymph nodes and inhibit their proliferation. Conclusion: Targeted arterial perfusion with paclitaxel liposome in combination with carboplatin is effective to reduce tumor size, attenuate the surgery induced injury and improve the post-operative quality of life of oral cancer patients.

Keywords: Paclitaxel, paclitaxel liposome, targeted arterial perfusion

Introduction

Oral cancer is one of the most common oral and maxillofacial malignancies and has an increasing incidence [1-5]. Patients with oral cancer usually have a low survival rate. Biologically, oral cancer is characterized by potent regional invasion and susceptibility to regional lymph node metastasis. In the present study, rabbit VX-2 tongue cancer model was established, and paclitaxel combined with carboplatin was administered via the lingual artery or ear vein, aiming to investigate the effects of these therapies on the apoptosis and proliferation of cancer cells in the primary cancer and metastatic lymph nodes and to screen better chemotherapeutics and a better way in which chemotherapeutics are administered. Our findings may provide experimental basis and theoretical evidence for the pre-operative adjuvant therapy of oral cancer.

Materials and methods

Materials

VX-2 tumor-bearing rabbits were kindly provided by the Central Laboratory of Sun Yat-sen University. A total of 42 New Zealand white rabbits aged 2-3 months and weighing 1.5-2.0 kg were purchased from the Experimental Center of Institute of Chinese Medicine in Chongqing (License No: SCXK [Yu] 2012-0006). Paclitaxel liposome injection (Nanjing LUYE PHARMA Co., Ltd), Paclitaxel injection (Harbin Pharmaceutical Group Bioengineering Co., Ltd), carboplatin injection (Qilu Pharmaceutical Co., Ltd), apoptosis detection kit (BD, USA), rabbit anti-P53 antibody (Beijing Bioss Biotech Co., Ltd), anti-rabbit secondary antibody (Beijing Bioss Biotech Co., Ltd) and 5% glucose (Sichuan Kelun Pharmaceutical Co., Ltd) were used in the present study.

Preparation of VX-2 cancer blocks

The VX-2 tumor-bearing rabbits aged 3 weeks were anesthetized intramuscularly with Lumianning II at 0.2 ml/kg. Then, animals were placed in a prone position and the cancers at the root of legs were carefully removed under an aseptic condition and washed in normal saline. The cancers were cut into blocks (1 mm^3 in size).

Rabbit VX-2 tongue cancer model

A total of 42 New Zealand rabbits were routinely anesthetized and the mouth was opened with a mouth gag. The tongue was pulled out of the mouth. A horizontal incision (2-3 mm) was made at the 1/3 site of lateral tongue, and the mucosa and muscle were separated, followed by implantation of 2-3 cancer blocks. The wound was carefully closed. Procaine penicillin (22000 U/kg) was intramuscularly injected within 3 days after surgery for the prevention of infection.

Grouping

VX-2 tumor-bearing rabbits with lymph node metastasis (n = 24) were randomly assigned into 6 groups (n = 4 per group). Lymph node metastasis was confirmed by color Doppler imaging examination. In A, B and C groups, rabbits received arterial perfusion of chemotherapeutics; in D, E and F groups, rabbits received venous perfusion of chemotherapeutics. Rabbits underwent food deprivation for 8 h before surgery and then were anesthetized. After skin preparation at the neck, rabbits in A, B and C groups were placed in a supine position and a midline incision (2.0 cm in length) was longitudinally made at the neck, and the fascia and muscles before the trachea were exposed. The common carotid artery and vagus nerve were identified along the paratracheal muscle gap, and the external carotid artery was identified above these tissues. The external carotid artery was ligated at the site proximal to the heart and retracted with a suture at the site distal to the heart. An oblique incision (0.5-1 mm) was made at the external carotid artery between two sutures, followed by insertion of an epidural catheter. The direction and depth of catheter were adjusted and methylene blue solution was injected via the catheter. The half tongue and tumor were stained, suggesting the catheter being inserted into the artery. The catheter was fixed in the external carotid artery with the second suture and then connected to a microinjection pump). In D, E and F groups, an infusion tube was inserted into the ear vein and then connected to a microinjection pump.

Grouping and treatments

The dose of chemotherapeutics (paclitaxel and carboplatin) was calculated according to dose conversion table between animals and humans. The dose of paclitaxel was 7.5 mg/kg and the dose of carboplatin was 13.0 mg/kg. Group A (VX-2 tumor-bearing rabbits treated with paclitaxel liposome and carboplatin via tongue arterial perfusion): Perfusion with 5% GS (20 ml) and L-PAC (11.25 mg) was completed within 30 min via the tongue artery; Perfusion with 5% GS (20 ml) and I-CAR (19.5 mg) was completed within 30 min via the tongue artery. Group B (VX-2 tumor-bearing rabbits treated with free paclitaxel and carboplatin via tongue arterial perfusion): Perfusion with 5% GS (20 ml) and L-PAC (11.25 mg) was completed within 30 min via the tongue artery; Perfusion with 5% GS (20 ml) and I-CAR (19.5 mg) was completed within 30 min via the tongue artery. Group C (VX-2 tumor-bearing rabbits treated with 5% glucose via tongue arterial perfusion): Perfusion with 5% GS (40 ml) was completed within 1 h via the tongue artery. Group D (VX-2 tumor-bearing rabbits treated with paclitaxel liposome and carboplatin via ear venous perfusion): Perfusion with 5% GS (20 ml) and L-PAC (11.25 mg) was completed within 30 min via the ear vein; Perfusion with 5% GS (20 ml) and I-CAR (19.5 mg) was completed within 30 min via the ear vein. Group E (VX-2 tumor-bearing rabbits treated with free paclitaxel and carboplatin via ear venous perfusion): Perfusion with 5% GS (20 ml) and L-PAC (11.25 mg) was completed within 30 min via the ear vein; Perfusion with 5% GS (20 ml) and I-CAR (19.5 mg) was completed within 30 min via the ear vein. Group F (VX-2 tumor-bearing rabbits treated with 5% glucose via ear venous perfusion): Perfusion with 5% GS (40 ml) was completed within 1 h via the ear vein.

Tumor growth and imaging examination

Four tumor-bearing rabbits were selected for the observation of tumor growth and survival time. At 2 weeks after implantation of cancer blocks, color Doppler imaging examination was done at the submandibular region, submental

Figure 1. Three weeks after implantation of cancer blocks, the cancers showed invasive growth, grew into the contralateral tongue and became ulcerous and hemorrhagic.

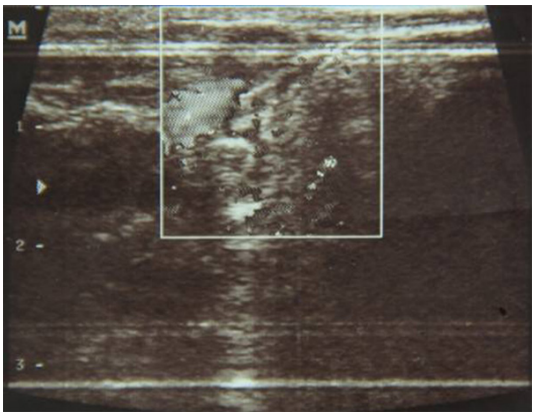

Figure 2. Color Doppler Ultrasonography of metastatic lymph nodes in rabbits with VX-2 cancer at the neck. Color Doppler Ultrasonography of neck lymph nodes showed lymph node enlargement (about 10 mm × 5 mm); the neck lymph nodes had unclear boundaries and were oval; hypoechoes were found at the center and hyperechoes of the medulla were narrowed, the echoes of the cortex became asymmetry and thickened, and net-like color, flow signals were observed in the lymph nodes.

region and neck, aiming to determine the lymph node metastasis. Criteria for lymph node metastasis: lymph nodes enlarged, the diameter was larger than 5 mm [6], the boundary of these lymph node was unclear, round, oval or irregular, the ratio of long diameter to short diameter was lower than 2, the lymph nodes showed uneven hypoechoic inside, the hyperechoic at the central medulla narrowed, the echoes of the cortex were asymmetry and thickened, and net-like, color flow signals were observed in the lymph nodes [7].

HE staining

After implantation of cancer blocks, color Doppler imaging examination was performed to detect the lymph node metastasis. The metastatic lymph nodes were collected from the neck and the cancers from the tongue for HE staining.

Transmission electron microscopy

Seven days after chemotherapy, the metastatic lymph nodes were collected from the neck and cut into blocks (about 1 mm^3 in size). The lymph node tissues were then fixed in 3% glutaraldehyde, followed by processing for transmission electron microscopy according to manufacturer's instructions.

Flow cytometry and immunohistochemistry

Seven days after chemotherapy, the primary cancers and metastatic lymph nodes were collected and washed in PBS to remove blood. The cancers were divided at the center and lymph nodes were separated along the long diameter. A half of cancers and lymph nodes were placed in serum free RPMI 1640 for subsequent detection of apoptosis by flow cytometry; the remaining part of cancers and lymph nodes were fixed in 4% paraformaldehyde and processed for immunohistochemistry for P53 according to manufacturer's instructions.

Statistical analysis

Data are expressed as mean ± standard deviation ($\bar{x} \pm s$) and comparisons were done with analysis of variance. Paired comparison was performed with LSD-t test. A value of $P < 0.05$ was considered statistically significant. Statistical analysis was performed with SPSS version 17.0.

Results

Observation after implantation of VX-2 cancer blocks

General activity and survival: Implantation of cancer blocks was done at 1/3 of the tongue of 42 rabbits, and VX-2 cancer was observed in 34 rabbits with the tumor formation rate of 80%. The cancers did not disappear spontaneously.

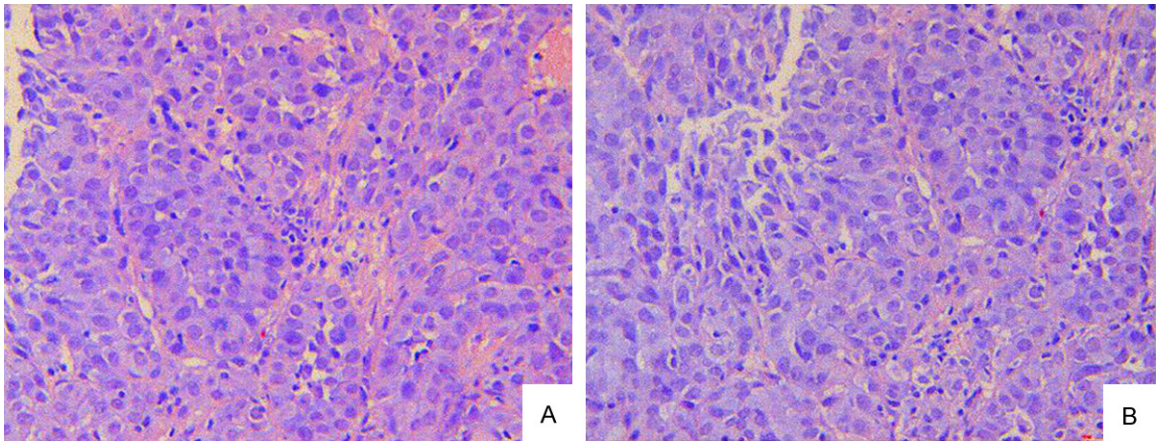

Figure 3. Pathological examination of the primary cancer and metastatic lymph nodes collected from rabbits with VX-2 squamous cancer. A, B: HE staining of primary VX-2 squamous cancer and metastatic lymph nodes (× 400) showed poorly differentiated cells, the morphology and structure of these cells became atypical and abnormal karyokinesis was observed.

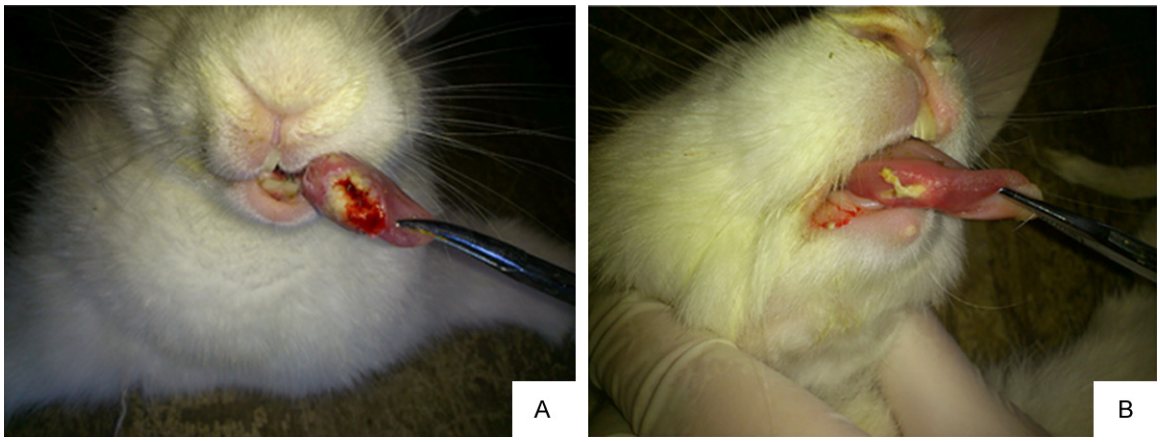

Figure 4. Primary VX-2 cancer before and after chemotherapy in Group A. A: Before chemotherapy, the tongue had processes, hard masses with different size were observed in the tongue, and some masses became ulcerous and were susceptible to bleed at touching; B: After chemotherapy, the tongue cancer shrunk significantly, the ulcer became healing and white pseudomembrane covered the cancer.

Tongue cancer: 1-4 weeks after implantation, the VX-2 cancer showed invasive and progressive growth (**Figure 1**). 4 weeks after implantation, the cancers involved the whole tongue and the cancer volume reached a maximum level. At 5-7 weeks, the cancers became ulcerous and necrotic due to the anatomical restrictions.

Lymph nodes of the neck: 30 tumor rabbit has 24 only color Doppler imaging examination showed lymph node enlargement and metastasis at 3-5 weeks after implantation of cancer blocks (**Figure 2**) with the lymph node metastasis rate of 80%. Metastasis was mainly found at the deep upper and middle cervical lymph nodes as well as submandibular lymph nodes. The tongue VX-2 cancer had the biological behaviors and metastatic sites similar to those seen in patients with tongue cancer.

HE staining: 4 weeks after implantation of cancer blocks, the primary cancer and metastatic lymph nodes of the neck were collected for HE staining. Squamous cancer was confirmed (**Figure 3**).

Evaluation of chemotherapeutic efficacy

Observation after chemotherapy: In arterial perfusion groups, tongue arterial insertion of catheter was successfully performed in 12 rabbits, and the cancer and affected tongue were

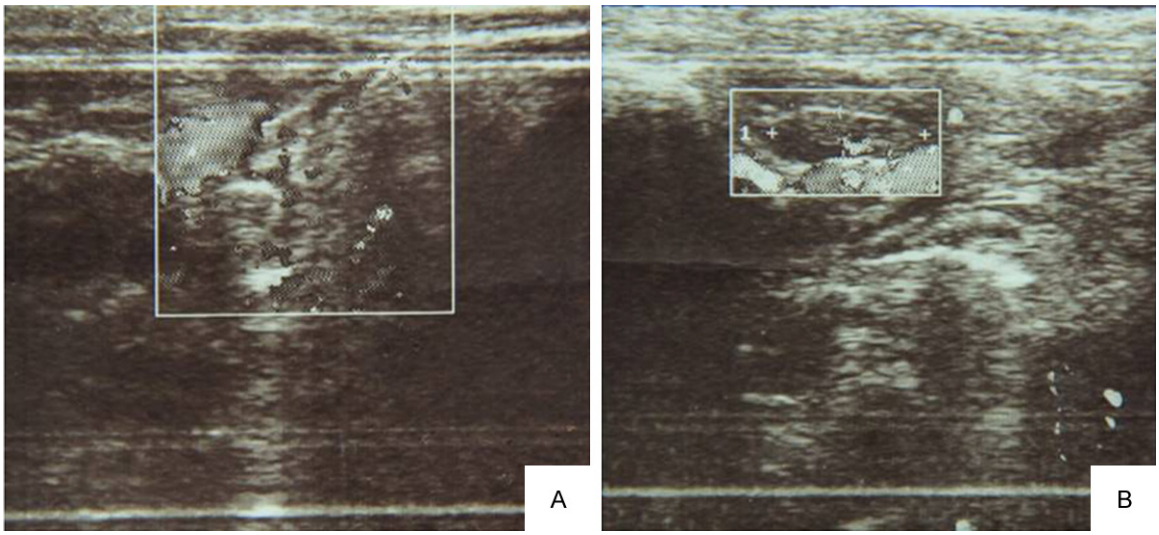

Figure 5. Neck lymph nodes before and after chemotherapy in Group A. A: Before chemotherapy, the lymph node size was about 10.0 mm × 5.0 mm; B: After chemotherapy, the lymph node size was about 9.7 mm × 3.0 mm.

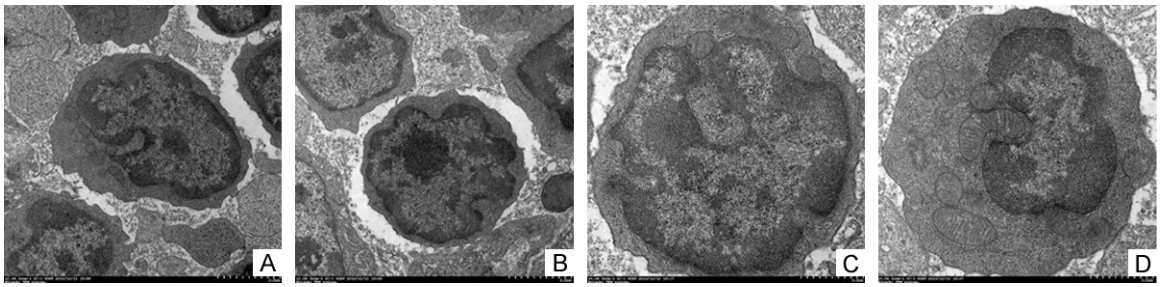

Figure 6. TEM showed the non-apoptotic cancer cells had large and abnormal nuclei, the nucleoli were large, and there were highly-developed mitochondria in the cytoplasm (A, B: × 2 K); Apoptotic cancer cells presented with cell shrinkage, cytoplasm condensation, intact cell membrane, karyopyknosis, formation of crescent-shaped nucleus, chromatin condensation and margination, absence of nucleoli and mitochondrial swelling (C, D: × 4 K).

Table 1. Apoptosis rate of cancer cells determined by flow cytometry at 1 week (X ± S, n = 12)

Group	n	Apoptosis rate (%)	F	*P*
A	12	84.80 ± 3.35$^{*,▲}$		
B	12	62.70 ± 7.92$^{*,\#}$		
D	12	52.05 ± 3.96$^{•,▲}$	586.364	$P < 0.05$
E	12	29.45 ± 0.15$^{•,\#}$		
C	12	8.63 ± 0.38		
F	12	8.61 ± 0.28		

Footnotes: *A vs. B, $P < 0.01$; ▲A1 vs. D, $P < 0.01$; #B vs. E, $P < 0.01$; •D vs. E, $P < 0.01$.

stained blue. Following chemotherapy, the rabbits had a poor spirit, slow movement and reduced appetite, but the vital signs were stable. At 7 days after chemotherapy, the primary cancer became shrunk, the superficial ulcer of the tongue became healing, and the tongue was not susceptible to bleeding after touching, covered with white pseudomembrane and soft at palpation (**Figure 4**). In Group A, ultrasonography of lymph nodes at the neck showed lymph nodes became smaller when compared with that before chemotherapy (**Figure 5**).

Transmission electron microscopy: TEM showed the number of apoptotic cancer cells was smaller in the lymph nodes of Group C and Group F, the cancer cells presented with large and abnormal nuclei, the nucleoli were large, several nuclei were observed in one cell, and highly-developed mitochondria and endoplasmic reticulum were observed; In Group B, Group D and Group E, apoptotic cancer cells were found in the lymph nodes to different extents;

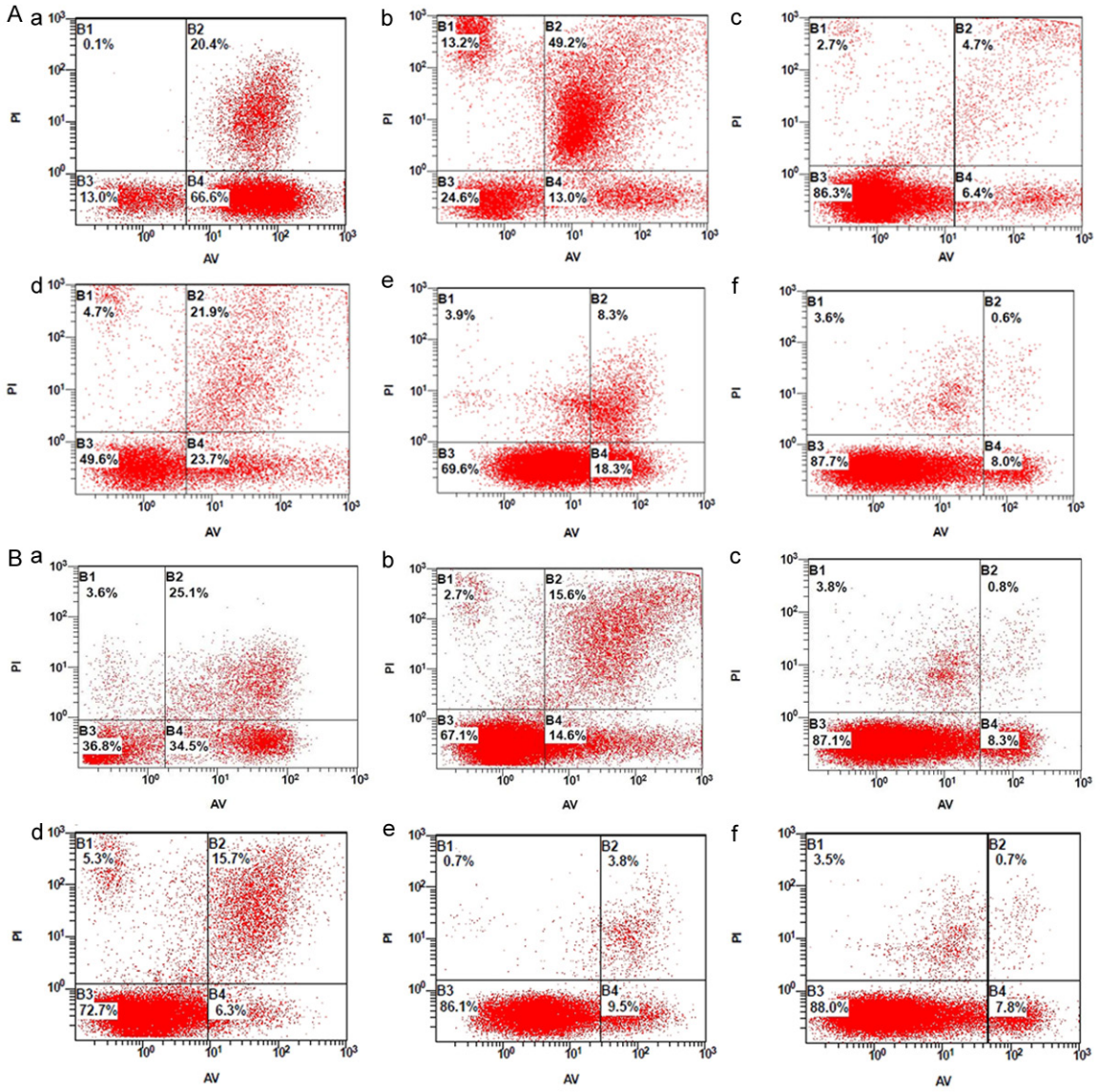

Figure 7. A. Flow cytometry of apoptotic cells in the primary cancers. a: Group A; b: Group B; c: Group C; d: Group D; e: Group E; f: Group F; B, Apoptosis rate of cancer cells in the metastatic lymph nodes determined by flow cytometry. a: Group A; b: Group B; c: Group C; d: Group D; e: Group E; f: Group F.

Group A: a large amount of cancer cells became apoptotic and they were characterized by cell shrinkage, intact membrane structure, karyopyknosis, chromatin margination, absence of nucleolus and presence of crescent-shaped nucleus (**Figure 6**).

Flow cytometry

In the primary cancers, the apoptosis rate was 84.80 ± 3.35% in Group A, 62.70 ± 7.92% in Group B, 8.63 ± 0.38% in Group C, 52.05 ± 3.96% in Group D, 29.45 ± 0.15% in Group E, and 8.61 ± 0.28% in Group F. Statistical analysis showed marked difference among these groups ($P < 0.05$) (**Table 1**; **Figure 7A**).

In the metastatic lymph nodes, the apoptosis rate was 49.49 ± 4.96% in Group A, 29.65 ± 3.61% in Group B, 8.59 ± 0.28% in Group C, 21.05 ± 1.65% in Group D, 12.98 ± 0.75% in Group E and 8.47 ± 0.30% in Group F (**Figure 7B**). Analysis of variance and LSD-t test showed significant difference ($P < 0.05$) except between Group C and Group F (**Table 2**). The apoptosis rate followed the following order: Group A, Group B, Group D, Group E, Group C and Group F.

Table 2. Apoptosis rate of cancer cells in the metastatic lymph nodes determined by flow cytometry at 1 week

Group	n	95% *CI* (%)	Apoptosis rate (%, $\bar{x} \pm s$)
A	4 × 3	46.34-52.64	49.49 ± 4.96*
B	4 × 3	27.35-31.94	29.65 ± 3.61*,☆
C	4 × 3	8.42-8.77	8.59 ± 0.28*,☆,•,#
D	4 × 3	20.00-22.10	21.05 ± 1.65*,☆,•,◊
E	4 × 3	12.50-13.46	12.98 ± 0.75*,•,◊,▲
F	4 × 3	8.28-8.66	8.47 ± 0.30*,☆,◊,▲,#

Footnote: *A vs. B; A vs. C; A vs. D; A vs. E; A vs. F; $P < 0.01$; ☆B vs. C, B vs. D, B vs. E, B vs. F, $P < 0.01$; •C vs. D, C vs. E, $P < 0.01$; ◊D vs. E, D vs. F, $P < 0.01$; ▲E vs. F, $P < 0.01$.

Immunohistochemistry

The P53 expression was 8.33% in Group A, 33.33% in Group B, 58.33% in Group D, 66.67% in Group E, 83.3% in Group C, and 83.33% in Group F. The P53 expression in Group A was significantly lower than that in other groups ($P < 0.05$), the P53 expression in Group B was also lower than that in other groups except for Group A, but no marked difference was observed among remaining groups ($P > 0.05$) (**Table 3**). The P53 protein expression was 75% in Group C and Group F, 20% in Group A, 33.33% in Group B, 41.67% in Group D and 50% in Group E. The positive rate in Group A was significantly lower than that in Group C and Group F ($P < 0.05$) (**Table 4**). No marked difference was observed among the remaining groups ($P > 0.05$).

Discussion

Oral cancer is one of the most common oral and maxillofacial - head and neck malignancies and frequently found in the tongue, cheek, the floor of the mouth and the gums. Squamous cell carcinoma accounts for 90% of head and neck malignancies [8], is highly invasive and usually predicts a low survival rate. Lymph vessels and blood vessels are the main routes of metastasis of head and neck squamous cell carcinoma, and neck is the most common site of metastasis of this cancer. The presence of neck lymph node metastasis often predicts a poor survival [9]. To investigate the pathogenesis and explore effective strategies for the prevention and therapy of oral cancer are very important. The comprehensive therapy is preferred for the management of oral cancer, including surgery, radiotherapy and chemotherapy. However, surgical intervention usually causes facial defects and dysfunction as well as a poor quality of life. Pre-operative chemotherapy may reduce the cancer size and narrow the extent of surgical resection, to increase the quality of life. Dünne [10] and Sapundzhiev [11] implanted VX-2 cancer at the ears of rabbits, and metastatic lymph nodes were observed, and the characteristics of VX-2 cancer were similar to those seen in humans. Jefferis [12] for the first time reported the establishment of tongue VX-2 squamous cell carcinoma model in rabbits, and the proportion of cancers collected was about 92.6%. Click [13] also established cheek VX-2 squamous cell carcinoma model in rabbits, and results showed cancer cells in the neck lymph nodes. In the present study, VX-2 cancer blocks were implanted in the tongue of rabbits to establish neck lymph node metastasis model, and results showed the biological characteristics of VX-2 squamous cell carcinoma were similar to those observed in human squamous cell carcinoma.

There is evidence showing that selective arterial perfusion of chemotherapeutics may improve the sensitivity of head and neck cancers. Under this condition, chemotherapeutics may more precisely reach the target organs as compared to intravenous perfusion and be more widely diffused as compared to intratumor injection of chemotherapeutics. Moreover, this strategy has few side effects as compared to systemic perfusion [14-19]. The response of cancers to chemotherapeutics depends on not only the sensitivity of cancer cells to chemotherapeutics, but the concentration and duration of chemotherapeutics in the cancers. In the chemotherapy via super-selective arterial catheterization, the focal concentration of chemotherapeutics was 4-22 times higher than that of the blood [20] Arterial perfusion of chemotherapeutics may maximize the concentration of drugs in the cancers, which reduces the cancer size, increases the chance to surgery, and reduces the surgery induced injury. When compared with systemic administration of chemotherapeutics, arterial perfusion reduces the systemic concentration of drugs, which avoids the side effects of systemic chemotherapy and then increases the quality of life.

Table 3. P53 expression in the VX-2 tongue cancer of rabbits at 1 week after chemotherapy

Group	n	P53				Number of positive expression	Positive rate %
		-	+	++	+++		
A	12	11	1	0	0	1	8.33▲,•,☆,○
B	12	8	2	2	0	4	33.33○
D	12	5	2	4	1	7	58.33•
E	12	4	3	3	2	8	66.67▲
C	12	2	2	4	4	10	83.33☆
F	12	2	3	2	5	10	83.33

Footnote: ▲A vs. E, $P < 0.05$; •A vs. D, $P < 0.01$; ☆A vs. C, A vs. F, $P < 0.05$.

Table 4. P53 expression in the metastatic lymph nodes at 1 week after chemotherapy

Group	P53 protein expression				Positive rate (%)	χ^2	P
	-	+	++	+++			
A	10	1	1	0	20.00*		
B	8	2	2	0	33.33		
C	3	3	4	2	75.00*	12.954	0.024
D	7	2	2	1	41.67		
E	6	3	1	2	50.00		
F	3	2	5	2	75.00*		

Footnote: *A vs. C, $P < 0.05$; A vs. F, $P < 0.05$.

Anti-cancer drugs are used to mainly inhibit the growth of cancer cells and induce their apoptosis. Apoptosis and division determine the occurrence and development of cancers and are controlled by numerous genes and enzymes (such as P5, Bcl-2/Bax, Fas/FasL, Caspases, c-myc and telomerase. Chemotherapeutics may alter the expressions of above genes to inhibit the proliferation of cancer cells and induce their apoptosis [27]. rAd-p53 is a drug used for gene therapy of cancers developed in recent years. Intratumor injection or selective arterial perfusion of wtP53 may significantly inhibit the proliferation of cancer cells and induce their apoptosis [28]. For patients with advanced oral cancer or other head and neck squamous cell cancers, rAd-p53 in combination with radiotherapy and/or chemotherapy has been found to increase the overall effectiveness and survival rate [29].

Paclitaxel is an effective broad-spectrum anti-cancer drug and has good effectiveness in some malignancies including lung cancer and breast cancer [21]. Paclitaxel has a poor solubility in water. In the help of polyoxyethylated castor oil and absolute ethanol, paclitaxel is soluble in water. However, this solution may cause severe side effects such as allergy and neurotoxicity [22]. Paclitaxel liposome (PTX-LP) significantly reduces the incidence of allergies in patients, which increases the patients' tolerance. PTX-LP is a new formula of paclitaxel prepared on the basis of liposome and studies have shown that PTX-LP has few side effects, but possesses anti-cancer effects as seen in paclitaxel [23]. PTX-LP has good cell affinity and histocompatibility, slow release capability, low toxicity and favorable stability, and thus it is an ideal drug carrier [24]. Platinum derivatives are also effective chemotherapeutics used in the therapy of head and neck squamous cell carcinoma [25]. Carboplatin, as a representative of second generation of platinum derivatives, has the anti-cancer characteristics of cisplatin, but possesses fewer side effects. Carboplatin has been widely applied in the therapy of small cell lung cancer and head and neck squamous cell carcinoma [26].

In the present study, flow cytometry showed the apoptosis rate of primary cancer was 84.80 ± 3.35% in Group A, 62.70 ± 7.92% in Group B, 8.63 ± 0.38% in Group C, 52.05 ± 3.96% in Group D, 29.45 ± 0.15% in Group E and 8.61 ± 0.28% in Group F. Significant difference was observed between Group A and Group D as well as between Group B and Group E ($P < 0.05$). After chemotherapy, the P53 protein expression 8.33% in Group A, 33.33% in Group B, 83.33% in Group C, 58.33% in Group D, 66.67% in Group E and 83.33% in Group F. This suggests that arterial perfusion with chemotherapeutics is more effective to induce apoptosis of cancer cells and to reduce P53 protein expression as compared to intravenous perfusion. In addition, when compared with free paclitaxel and carboplatin, paclitaxel liposome combined with carboplatin was more effective to induce the apoptosis of cancer cells and down-regulate P53 protein expression.

Flow cytometry showed the apoptosis rate was 49.49 ± 4.96% in Group A, 29.65 ± 3.61% in Group B, 8.59 ± 0.28% in Group C, 21.05 ± 1.65% in Group D, 12.98 ± 0.75% in Group E and 8.47 ± 0.30% in Group F. This suggests that arterial perfusion of chemotherapeutics

achieved a better therapeutic efficacy as compared to intravenous perfusion, and paclitaxel liposome combined with carboplatin were superior to free paclitaxel in combination with carboplatin in the therapeutic efficacy. The P53 protein expression was 20% in Group A, 33.33% in Group B, 75% in Group C, 41.67% in Group D, 50% in Group E and 75% in Group F. The P53 expression in Group A was significantly lower than that in Group C and Group F ($P < 0.05$), suggesting the proliferation of cancer cells in Group A was effectively inhibited. This implies that to down-regulate P53 expression might be a mechanism underlying the chemotherapy (arterial perfusion of paclitaxel liposome combined with carboplatin) induced apoptosis of cancer cells in the metastatic lymph nodes of rabbits with tongue VX-2 squamous cell cancer.

In the present study, VX-2 tongue squamous cell cancer with neck lymph node metastasis is successfully established in rabbits in the present study. Our results demonstrate that VX-2 cancer in rabbits has biological characteristics similar to those seen in patients. In addition, arterial perfusion with paclitaxel liposome combined with carboplatin is more effective to induce the apoptosis of cancer cells and inhibit their proliferation in the primary cancer and metastatic lymph nodes as compared to perfusion via the ear vein. We speculate that arterial perfusion with paclitaxel liposome combined with carboplatin may significantly reduce the cancer size and reduce the extent of surgical resection, which makes the preservation of morphology and function of organs possible. Moreover, this therapeutic strategy also reduces the surgery induced injury and increases the post-operative quality of life. Thus, arterial perfusion of chemotherapeutics is recommended as a strategy for pre-operative induction chemotherapy.

Acknowledgements

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Disclosure of conflict of interest

None.

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References

[1] Parkin DM, Bray F, Ferlay J and Pisani P. Global cancer statistics, 2002. CA Cancer J Clin 2005; 55: 74-108.

[2] Mayne ST, Morse DE and Winn DM. Cancers of the oral cavity and pharynx. In: Schottenfeld D, Fraumeni JF, editors. Cancer epidemiology and Prevention. New York: Oxford University Press; 2006. pp. 674-696.

[3] Melrose RJ. Premalignant oral mucosal diseases. J Calif Dent Assoc 2001; 29: 593-600.

[4] King M, Chatelain K, Farris D, Jensen D, Pickup J, Swapp A, O'Malley S and Kingsley K. Oral squamous cell carcinoma proliferative phenotype is modulated by proanthocyanidins: a potential prevention and treatment alternative for oral cancer. BMC Complement Altern Med 2007; 7: 22.

[5] Ragin CC, Modugno F and Gollin SM. The epidemiology and risk factors of head and neck cancer: a focus on human papillomavirus. J Dent Res 2007; 86: 104-114.

[6] Ling R, Li Y, Yao Q, Chen T, Zhu D, Jun Y and Chen J. Lymphatic chemotherapy induces apoptosis in lymph node metastases in a rabbit breast carcinoma model. J Drug Target 2005; 13: 137-142.

[7] Vassallo P, Wernecke K, Roos N and Peters PE. Differentiation of benign from malignant superficial lymphadenopathy: the role of high-resolution US. Radiology 1992; 183: 215-220.

[8] Cooper JS, Porter K, Mallin K, Hoffman HT, Weber RS, Ang KK, Gay EG and Langer CJ. National Cancer Database report on cancer of the head and neck: 10-year update. Head Neck 2009; 31: 748-758.

[9] Koischwitz D and Gritzmann N. Ultrasound of the neck. Radiol Clin North Am 2000; 38: 1029-1045.

[10] Dunne AA, Mandic R, Ramaswamy A, Plehn S, Schulz S, Lippert BM, Moll R and Werner JA. Lymphogenic metastatic spread of auricular VX2 carcinoma in New Zealand white rabbits. Anticancer Res 2002; 22: 3273-3279.

[11] Sapundzhiev N, Dunne AA, Ramaswamy A and Werner JA. The auricular VX2 carcinoma: feasibility of complete tumor resection. Anticancer Res 2005; 25: 4209-4214.

[12] Jefferis AF and Berenbaum MC. The rabbit VX2 tumour as a model for carcinomas of the tongue and larynx. Acta Otolaryngol 1989; 108: 152-160.

[13] Chikui T, Yuasa K, Maemura S and Kanda S. Change of angiostructure and hemodynamics in lymph node metastases in rabbits. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2002; 93: 350-357.
[14] Reid T, Warren R and Kirn D. Intravascular adenoviral agents in cancer patients: lessons from clinical trials. Cancer Gene Ther 2002; 9: 979-986.
[15] Robbins KT and Homma A. Intra-arterial chemotherapy for head and neck cancer: experiences from three continents. Surg Oncol Clin N Am 2008; 17: 919-933, xi.
[16] Fuwa N, Kodaira T, Furutani K, Tachibana H and Nakamura T. A new method of selective intra-arterial infusion therapy via the superficial temporal artery for head and neck cancer. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2008; 105: 783-789.
[17] Wu CF, Huang CJ, Chang KP and Chen CM. Continuous intra-arterial infusion chemotherapy as a palliative treatment for oral squamous cell carcinoma in octogenarian or older patients. Oral Oncol 2010; 46: 559-563.
[18] Fuwa N, Kodaira T, Furutani K, Tachibana H, Nakamura T, Nakahara R, Tomoda T, Inokuchi H and Daimon T. Intra-arterial chemoradiotherapy for locally advanced oral cavity cancer: analysis of therapeutic results in 134 cases. Br J Cancer 2008; 98: 1039-1045.
[19] Kovacs AF. Intra-arterial induction high-dose chemotherapy with cisplatin for oral and oropharyngeal cancer: long-term results. Br J Cancer 2004; 90: 1323-1328.
[20] Cui P, Yu M, Peng X, Dong L and Yang Z. Melatonin prevents human pancreatic carcinoma cell PANC-1-induced human umbilical vein endothelial cell proliferation and migration by inhibiting vascular endothelial growth factor expression. J Pineal Res 2012; 52: 236-243.
[21] Ferlini C, Gallo D and Scambia G. New taxanes in development. Expert Opin Investig Drugs 2008; 17: 335-347.
[22] Koudelka S and Turanek J. Liposomal paclitaxel formulations. J Control Release 2012; 163: 322-334.
[23] Wang H, Cheng G, Du Y, Ye L, Chen W, Zhang L, Wang T, Tian J and Fu F. Hypersensitivity reaction studies of a polyethoxylated castor oil-free, liposome-based alternative paclitaxel formulation. Mol Med Rep 2013; 7: 947-952.
[24] Nie S, Hsiao WL, Pan W and Yang Z. Thermoreversible Pluronic F127-based hydrogel containing liposomes for the controlled delivery of paclitaxel: in vitro drug release, cell cytotoxicity, and uptake studies. Int J Nanomedicine 2011; 6: 151-166.
[25] Volling P, Schroder M, Rauschning W, Achterrath W and Stennert E. Carboplatin. The better platinum in head and neck cancer? Arch Otolaryngol Head Neck Surg 1989; 115: 695-698.
[26] Owonikoko TK, Ramalingam SS, Kanterewicz B, Balius TE, Belani CP and Hershberger PA. Vorinostat increases carboplatin and paclitaxel activity in non-small-cell lung cancer cells. Int J Cancer 2010; 126: 743-755.
[27] Sinicrope FA, Hart J, Hsu HA, Lemoine M, Michelassi F and Stephens LC. Apoptotic and mitotic indices predict survival rates in lymph node-negative colon carcinomas. Clin Cancer Res 1999; 5: 1793-1804.
[28] Zhang L, Yu D, Hu M, Xiong S, Lang A, Ellis LM and Pollock RE. Wild-type p53 suppresses angiogenesis in human leiomyosarcoma and synovial sarcoma by transcriptional suppression of vascular endothelial growth factor expression. Cancer Res 2000; 60: 3655-3661.
[29] INGN 201: Ad-p53, Ad5CMV-p53, adenoviral p53, p53 gene therapy-introgen, RPR/INGN 201. Drugs R D 2007; 8: 176-187.