ORIGINAL ARTICLE

Dosimetry study on radioactive particle brachytherapy in oral carcinoma

Zhang Jie, Meng Jian, Zhuang Qianwei, Li Zhiping

Department of Stomatology, Xuzhou Central Hospital, Xhuzhou City, China

Summary

Purpose: *To investigate the therapeutic effects of different doses of* ^{125}I *radioactive particle brachytherapy on oral cancer.*

Methods: *Between September 2012 and September 2015, 78 patients with oral cancer who received* ^{125}I *radioactive particle brachytherapy for the first time in our hospital were enrolled in this study. Patients were divided into high dose (≥3) and low dose (<3) groups. The treatment outcome, serum tumor marker levels and the expression levels of autophagy and apoptotic genes in tumor cells were compared between groups.*

Results: *Complete remission (CR)+partial remission (PR) ratio in the high dose group was significantly higher than that of the low dose group. Stable disease (SD)+ progressive disease (PD) ratio was significantly lower in the high dose group. The serum levels of TSGF, SCCA, CEA, CA125,*

CA15.3, CA19.9 and PSA oral cancer markers were significantly lower than those of the low dose group. In the high dose group, the expression levels of Beclin-1 and MAP1LC3 (autophagic genes) mRNAs were significantly higher than those of the low dose group, while the expression levels of EMMPRIN and MMP-14 (invasive genes) mRNAs were significantly lower in the high dose group. Also survival rates in the responsive patients were significantly better in comparison to non-responsive patients.

Conclusion: *High dose particle brachytherapy with radioactive* ^{125}I *is a safe and effective treatment and its clinical results were more beneficial than the low dose therapy.*

Key words: *apoptosis, brachytherapy, dose, oral carcinoma/oral cancer*

Introduction

More than 90% of oral carcinomas are of squamous cell histology [1]. It has been shown that in prostate and liver cancers, elderly patients and those with poor health who cannot tolerate surgery and radiotherapy, can be treated with radioactive particle brachytherapy [1,2]. Brachytherapy places radioactive sources inside the tumor to damage cancer cells' DNA and destroy their ability to divide and grow [3]. ^{125}I radioactive particle can constantly release low doses of γ-ray. which causes cellular water molecules ionization and generates large quantities of free radicals which can seriously damage cancer cells' DNA [4]. A previous study [5] reported the use of ^{125}I radioactive

particle in the local treatment of oral cancer. In the present study, we investigated the effects of different doses of ^{125}I radioactive particle on the treatment of patients with oral cancer.

Methods

General information

Between September 2012 and September 2015, 78 patients with oral cancer who received radioactive particle brachytherapy for the first time in our hospital were continuously enrolled in this study. Inclusion criteria: (i) patients with primary oral cancer confirmed by biopsy; (ii) patients who received treatment for the

first time; (iii) patients with predicted survival greater than 6 months; and (iv) patients unsuitable for surgery. Exclusion criteria: (i) concomitant with primary tumor from other tissues and organs; (ii) patients with severe organ dysfunction including heart, lung, liver and kidneys and unable to tolerate surgery; (iii) patients with abnormal coagulation; and (iv) patients with mental disorders and those unable or unwilling to cooperate.

The mean number of intraoperative radioactive particles was calculated and patients were divided into the high dose group (≥3) and the low dose group (<3) with 39 patients in each group.

Study methods

Radioactive particle implanting devices were used in both groups. 18G particle implanting needles and wheel implanting guns were used. The radioactive ^{125}I particle was 4.5 mm in length, 0.8 mm in diameter, with a half-life of 59.6 days and radioactivity of 0.6-0.8 mCi per particle. Intraoperative plain CT scan was performed to examine important peripheral oral organs (thickness 2 mm) including target volume. Puncture was guided under CT and the position and puncture point were selected based on planning target volume (PTV). Patients received conventional disinfection and target was directly reached by fan shaped arranging needle after local anesthesia in order to implant ^{125}I radioactive particles inside the tumor and 0.5 cm from its periphery. Particle interval was controlled between 0.5 to 1 cm. CT scan (thickness 2 mm) was performed immediately after implantation in order to verify whether the particles were shifted. The hemostatic medications or appropriate antibiotics were administered by intramuscular injection after operation. Particle application was well recorded and images were transferred into the treatment plan system of the computer for validation.

Evaluation criteria for treatment effects and outcome

In order to evaluate treatment effects and outcome, patients were re-examined with CT scan 6 months after treatment. CR was defined as complete disappearance of tumor in one month with no tumor detection during imaging examination. PR was defined as tumor shrinkage ≥ 50% lasting more than a month. If tumor shrinkage <50% or increase ≤ 25% after one month this was evaluated as SD. If tumor size increased by >25% or new lesion(s) appeared, this was evaluated as PD.

Tumor markers

Peripheral venous blood (5 ml) samples were collected after fasting. Samples were centrifuged, at 2500 g for 30 min. Supernatant was collected and tumor marker levels were measured with ELISA. Measured were the levels of tumor specific growth factor (TSGF), squamous cell carcinoma antigen (SCCA), carcinoembryonic antigen (CEA), carbohydrate antigen 125 (CA125), carbohydrate antigen 15.3 (CA15.3), carbohydrate antigen 19.9 (CA19.9) and prostate specific antigen (PSA).

Autophagy and invasive gene expression in tumor biopsy

Tissue samples were collected and total RNA was extracted with Trizol. cDNA was synthesized using reverse transcription kits. PCR reaction system was then conducted using cDNA. Primers were designed using data existed in Gene Bank and were constructed by Shanghai Biological Engineering Co., Ltd. PCR was carried out in accordance with established procedures (95 ºC for 10 s, 58 ºC for 5 s, 72 ºC for 20 s). The amplification curves were obtained after 40 cycles. The relative expression levels of Beclin-1 and MAP1-LC3 (autophagic genes) and EMMPRIN and MMP-14 (invasive genes) mRNAs were evaluated.

Liver function

Peripheral venous blood (2 ml) was collected after fasting. Samples were centrifuged, the supernatant was collected and liver function tests including total bilirubin (TBiL) and gamma-glutamyltransferase (GGT) levels and renal function tests including blood urea nitrogen (BUN) and creatinine (CRE) levels were performed.

Statistics

SPSS23.0 software was used for statistical analysis. For measurement data we used t test and for enumeration data we used chi-square test. A p value<0.05 was considered statistically significant.

Results

Grouping results

In the high dose group there were 21 males and 18 females, and the age ranged from 36 to 69 years (mean=54.38±6.11). Of the patients in the high dose group 13 had carcinoma of gingiva, 11 tongue cancer, 9 buccal mucosa cancer and 6 oral floor carcinoma. In the low dose group, there were 20 males and 19 females with age ranging from 34 to 72 years (mean=58.76±6.59).

In the low dose group 14 patients had gingival carcinoma, 10 tongue cancer, 10 buccal mucosa cancer, and 5 oral floor carcinoma. Differences in sex, age and types of tumor between two groups, were not statistically significant (p>0.05).

Post-treatment CR+PR ratio in the high dose group was higher than that of the low dose group (32/82.5% vs 24/61.53%), while SD+PD ratio was lower than that of the low dose group (7/17.95% vs 15/38.47%; p<0.05) (Table 1). Survival was also significantly higher in total response rates in both

Table 1. Comparison of treatment results 6 months after treatment between the two groups

Group	Cases, n	CR n (%)	PR n (%)	SD n (%)	PD n (%)
High dose group	39	20（51.28）	12（30.77）	6（15.38）	1（2.57）
Low dose group	39	15（38.45）	9（23.08）	11（28.21）	4（10.26）
x^2 4.052					
P 0.044					

For abbreviations see text

Table 2. Comparison of 5-year survival rate between non-responsive and total-responsive patients in the two groups

Groups	5-year survival rate (%)					
	Non-responsive patients			Total-responsive patients		
	Number	Survival	Survival rate (%)	n	Survival	Survival rate (%)
High dose group (n=39)	14	6	42.9	25	22	88.0
Low dose group (n=39)	18	8	44.4	21	16	76.2

Table 3. Comparison of serum tumor maker levels 6 months post-therapy between the two groups

Group	TSGF (μg/ml)	SCCA (μg/L)	CEA (ng/ml)	CA125 (U/ml)	CA15.3 (U/ml)	CA19.9 (U/ml)	PSA (μg/L)
High dose group	59.73±6.12	0.31±0.04	2.53±0.31	53.28±6.12	38.23±4.09	40.15±4.85	19.36±2.47
Low dose group	68.53±7.12	0.58±0.06	2.99±0.43	95.26±10.19	64.29±7.18	76.42±8.06	34.57±4.13
t	6.485	5.039	5.182	8.293	7.172	8.364	8.023
P	0.019	0.034	0.027	0.009	0.015	0.005	0.012

For abbreviations see text

Table 4. Bioptic tumor autophagic gene and invasive gene expression 6 months post-therapy

Group	Autophagic genes		Invasive genes
	Beclin-1	MAP1LC3	EMMPRIN
High dose group	153.29±17.63	162.18±19.54	74.28±8.11
Low dose group	100±9.23	100±10.74	100±9.62
t	7.293	8.973	7.394
P	0.023	0.009	0.019

For abbreviations see text

groups as compared to no response patients (Table 2). TSGF, SCCA, CEA, CA12.5, CA15.3, CA19.9 and PSA serum levels in the high dose group were lower than those of the low dose group. All differences were statistically significant (p<0.05) (Table 3).

Autophagic genes, Beclin-1 and MAP1LC3, mRNA expression levels in the high dose group were higher than those of the low dose group, while the mRNA expression levels of invasive genes, EMMPRIN and MMP-14, were lower than those of the low dose group. Differences were statistically significant (p<0.05) (Table 4).

TBiL, GGT, BUN and CRE levels did not show any significant difference (p>0.05) (Table 5).

Discussion

Patients are usually diagnosed when oral cancer is already in moderate or advanced stages. Often, surgical resection has limited effect and cannot eliminate metastatic lesions [6]. The application of ^{125}I radioactive particle brachytherapy in prostate cancer and liver cancer is a known method of treatment for these types of cancer. Results obtained from a previous study [7] revealed that ^{125}I radioactive particle implantation effectively prolonged the survival time in cancer patients. A similar study [8]

Table 5. Hepatic and renal function levels in the two groups 6 months post-therapy

Group	Hepatic function		Renal function	
	TBiL(μmol/L)	GGT(U/L)	BUN(mmol/L)	CRE(μmol/L)
High dose group	7.59±0.83	39.72±4.85	6.39±0.71	73.39±8.17
Low dose group	6.43±0.78	35.19±4.24	5.41±0.63	63.21±7.23
t	0.535	0.782	0.492	0.683
P	0.593	0.329	0.673	0.452

For abbreviations see text

showed that ^{125}I had an important destructive effect on tumor angiogenesis and invasive capabilities. The unified dose of radioactive particles in the treatment of malignant tumors is 95% of gross target volume (GTV) and can reach the prescription dose (PD). Moreover, the majority of authors believe that the GTV dose should not exceed 2PD in order to avoid damages to normal tissues. Establishing the right dose is the key for a successful therapy.

Results obtained from a previous study [9] revealed that TSGF, SCCA, CEA, CA125, CA15.3, CA19.9 and PSA were associated with the treatment outcome and relapse of oral cancer. In the present study we showed that the levels of all the abovementioned indexes were significantly lower after the treatment in the high dose group. TSGF is a factor related to malignant tumor growth and is considered an auxiliary index for broad spectrum malignant tumor detection. TSGF level has been shown to be positively correlated with the tumor [10]. CEA is a non-specific tumor index and its level may increase in malignant tumors. SCCA is an important auxiliary diagnostic factor in head and neck neoplasms. A previous study [11] showed that SCCA level may increase prior to tumor recurrence. CA125, CA15.3 and CA19.9 are the most common tumor markers in various tumors. Previous evidence [12] confirmed that CA125, CA15.3 and CA19.9 levels are important factors in the identification of benign and malignant tumors. Lately it has been reported that PSA is a highly specific tumor marker used for oral cancer diagnosis and was shown that its level increased with the progression of oral cancer [12].

Autophagy is a highly conservative cell behavior that represents a self-repair mechanism in which the defective or damaged cellular components are entrapped in a phagophore. Most of the chemotherapy-resistant cancers, including specific oral cancers, have demonstrated autophagy to evade cell death [13]. Detecting the expression of autophagy and invasion-related genes in tumor tissue is a reliable method that reflects the severity of malignant tumor and could evaluate the treatment outcome [13]. When a malignant tumor is forming, autophagy becomes abnormal and affects the tumor growth, proliferation and apoptosis, and directly influences the progression

of cancer [14]. Beclin-1 and MAP1LC3 are two typical autophagic genes. Beclin-1 is mammal's specific autophagic gene and MAP1LC3 participates in the modification of ubiquitin-like proteins. A recent study discovered that the autophagic genes Beclin-1 and MAP1LC3 were low-expressed in the gingival and tongue cancer tissue [15]. EMMPRIN and MMP-14 are typical invasion-related genes, and their high expressions are directly associated with angiogenesis and tumor cell invasion and metastasis [16]. Results obtained from the current study showed that, in the high dose group, the autophagic genes Beclin-1 and MAP1LC3 mRNA expression levels were significantly higher, while the autophagic genes EMMPRIN and MMP-14 mRNA expression levels were obviously lower. It is suggested that high dose of ^{125}I brachytherapy could constantly send up high power radiation to influence the peripheral tumor tissue activities and to activate its autophagic progress and suppress the tumor cell invasion and metastasis.

Currently, the use of high dose radioactive particle implantation as an efficient anticancer therapy is getting more popularity among researchers. The extent of radiation damage to peripheral normal tissue is still unknown, therefore some experts expressed their reservations about its safety [3,17]. Due to the fact that hepatic and renal function is highly sensitive to radiation damage, in this study, we compared hepatic and renal function indexes between the two groups. Our results showed no significant changes regarding liver and kidney function after high dose therapy compared with low dose therapy.

We conclude that high dose particle brachytherapy with radioactive ^{125}I is a safe and effective treatment and its effectiveness is superior compared with low dose brachytherapy.

Acknowledgement

Conflict of interests

The authors declare no conflct of interests.

References

1. Yung KW, Yung TT, Chung CY et al. Principles of cancer staging. Asian Pac J Surg Oncol 2015;1:1-16.

2. Cahill G, Tran L, Baker RA, Brown A, Huang YC, Ou CW. Principles of radiation oncology. Asian Pac J Surg Oncol 2015;1:27-38.

3. Stannard C, Maree G, Tovey S, Hunter A, Wetter J. Iodine-125 brachytherapy in the management of squamous cell carcinoma of the oral cavity and oropharynx. Brachytherapy 2014;13:405-412.

4. McCaul JA, Cymerman JA, Hislop S et al. LIHNCS - Lugol's iodine in head and neck cancer surgery: a multicentre, randomised controlled trial assessing the effectiveness of Lugol's iodine to assist excision of moderate dysplasia, severe dysplasia and carcinoma in situ at mucosal resection margins of oral and oropharyngeal squamous cell carcinoma: study protocol for a randomised controlled trial. Trials 2013;14:310.

5. McMahon J, Devine JC, McCaul JA, McLellan DR, Farrow A. Use of Lugol's iodine in the resection of oral and oropharyngeal squamous cell carcinoma.Br J Oral Maxillofac Surg 2010;48:84-87.

6. Zheng YF, Tan LK, Tan BH, Sterling H, Kane R. Principles of surgical oncology. Asian Pac J Surg Oncol 2015;1:17-26.

7. Romagna A, Schwartz C, Egensperger R et al. Iodine-125 brachytherapy as upfront and salvage treatment for brain metastases: A comparative analysis. Strahlenther Onkol 2016;6:145-146.

8. Magill ST, Lau D, Raleigh DR, Sneed PK, Fogh SE, McDermott MW. Surgical Resection and Interstitial Iodine-125 Brachytherapy for High-Grade Meningiomas: A 25-Year Series. Neurosurgery 2016;6:12-13.

9. Feng XY, Li JH, Li JZ, Han ZX, Xing RD. Serum SCCA, Cyfra 21-1, EGFR and Cyclin D1 levels in patients with oral squamous cell carcinoma. Int J Biol Markers 2010;25:93-98.

10. Wang G, Qin Y, Zhang J et al. Nipple discharge of CA15-3, CA125, CEA and TSGF as a new biomarker panel for breast cancer. Int J Mol Sci 2014;15:9546-9565.

11. Yoshida Y. Effective biological marker to detect oral squamous cancer cells--expression patterns of CK 10, 17, 19 and SCCA mRNA. Kokubyo Gakkai Zasshi 2007;1:37-42.

12. Haffner MC, Laimer J, Chaux A et al. High expression of prostate-specific membrane antigen in the tumor-associated neo-vasculature is associated with worse prognosis in squamous cell carcinoma of the oral cavity. Mod Pathol 2012;25:1079-1085.

13. Li YN, Hu JA, Wang HM. Inhibition of HIF-1α Affects Autophagy Mediated Glycosylation in Oral Squamous Cell Carcinoma Cells. Dis Markers 2015;2015:239479.

14. Tang JY, Fang YY, Hsi E et al. Immunopositivity of Beclin-1 and ATG5 as indicators of survival and disease recurrence in oral squamous cell carcinoma. Anticancer Res 2013;33:5611-5616.

15. Tang JY, Hsi E, Huang YC, Hsu NC, Chu PY, Chai CY. High LC3 expression correlates with poor survival in patients with oral squamous cell carcinoma. Hum Pathol 2013;44:2558-2562.

16. Siu A, Chang J, Lee C, Lee S, Lee C, Ramos DM. Expression of EMMPRIN modulates mediators of tumor invasion in oral squamous cell carcinoma. J Calif Dent Assoc 2013;41:831-838.

17. Yu UP, Yu Q, Guo JM, Jiang HT, Di XY, Zhu Y. [125]I particle implantation combined with chemoradiotherapy to treat advanced pancreatic cancer. Br J Radiol 2014;87:20130641.