Neoadjuvant Chemotherapy Followed by Limited Surgery in a Mouse Model of Head and Neck Cancer

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Abstract. Background: This study evaluated the effect of limited surgery following neoadjuvant chemotherapy compared to radical surgery in SCC VII (squamous cell cancer) tumor-bearing syngeneic C3H/HeJ mice. Materials and Methods: Mice showing a tumor response to cisplatin and 5-fluorouracil therapy were divided into three groups: radical surgery, with a 5 mm margin from the original tumor, group (A); limited surgery of 5 mm margin from the residual tumor, group (B); and very limited surgery with no margin from the residual tumor, group (C). The number of mice was 13 (group A), 12 (group B) and 12 (group C). Results: No recurrence developed except in one mouse in group C. Three mice died in group A, one in group B and one in group C from perioperative complications. By intent-to-treat analysis, the survival was not significantly different among the three groups (p=0.64), or between group A and B (p=0.33). Conclusion: The outcome of limited surgery was comparable to radical surgery after neoadjuvant chemotherapy in the mouse model of head and neck cancer.

Squamous cell cancer (SCC) of the head and neck is the sixth most common cancer and accounts for 3% of all new cancer cases worldwide, as well as in Asia (1, 2). The 5-year survival rate of locally advanced stages is around 50%, and the primary reason for treatment failure is locoregional recurrence (1, 3). Histopathological margins are considered to be one of the causes of locoregional recurrence (4, 5).

The anatomical characteristics of head and neck cancer are an infiltrative non-cohesive pattern, permeation of vessels and nerves, and submucosal spread (6-8). Moreover, skip micrometastasis may exist because of the discontinuous nature of head and neck cancer (9). Wide excision with more than 5 mm margins has generally been accepted in current practice as the goal of curative oncological surgery in order to remove all tumor and leave no residual neoplastic tissue (10). Some authors have insisted that a minimum 1.5 cm surgical margin should be recommended because of skip micrometastasis beyond the resection margin (9). However, obtaining a clear margin has been shown to be consistent with a lower rate of local recurrence but to be inconsistent with a higher rate of survival (11-13).

Head and neck cancer develops from the structures involved in speech, voice production and swallowing. Wide excision and radical surgery with the intent to cure, is associated with a high rate of complications, morbidities, loss of important functions and low quality of life (14-17). A major concern about wide excision is not only the functional and esthetic morbidity and mortality of surgery, but the possibility of promoting cancer growth by damage to immune control and by extending the delay in delivering adjuvant therapy (18).

The uncertainty of the surgical margin has been a concern with the development of a minimalist surgical approach using a very narrow surgical margin as well as neoadjuvant chemotherapy (19-22). Neoadjuvant chemotherapy with newly developing target agents has significant theoretical advantages (23-25) and a significant benefit has been observed with platinum-based neoadjuvant chemotherapy (hazard ratio 0.88, 95% CI 0.79-0.97) in a meta-analysis of neoadjuvant clinical trials (25). Therefore, perioperative chemotherapy has been utilized to improve the survival in locally advanced head and neck cancer. The nature and definition of tumor margins following neoadjuvant chemotherapy or chemoradiotherapy has been undefined. Even though determination of the optimal...
resection margin after neoadjuvant chemotherapy is important, it is ethically difficult to perform a randomized clinical trial in the human setting.

The purpose of this study was to investigate the efficacy of limited surgery based on the residual tumor compared to radical surgery following neoadjuvant chemotherapy in a tumor-bearing mouse model.

Materials and Methods

Cell line. SCC VII, a murine squamous carcinoma cell line, was maintained in complete media consisting of RPMI-1640 media (Life Technologies, Grand Island, NY, USA) supplemented with 10% fetal bovine serum and antibiotic-antimycotics (100 units/ml penicillin, 100 μg/ml streptomycin and 25 μg/ml amphotericin B), at 37˚C in a humidified atmosphere of 95% air and 5% CO₂. The SCC VII cells were passaged at the Massachusetts General Hospital (26).

Tumor-bearing mouse model and tumor implantation. For tumor development, SCC VII cells (10⁵-10⁷) in a 100-μl suspension were subcutaneously injected into the upper hind limbs of C3H/HeJ mice (Korea Biolink Co., Eumsung, Republic of Korea). Subsequently, SCC VII tumor tissue was obtained from the tumors of these donor C3H/HeJ mice. A tumor-bearing mouse model was developed by implanting SCC VII tumor tissue into syngeneic C3H/HeJ mice (male, 10-week old; Korea Biolink Co.). For the current study, a 2x2x2 mm segment of SCC VII tumor tissue was intramuscularly implanted into the buttock muscle of each C3H/HeJ syngeneic mouse. All the experiments were performed with the authorization of the Animal Experiment Committee at the Clinical Research Institute of Seoul National University Hospital.

Anticancer chemotherapy. When a 10x10 mm tumor mass developed, the mice were tattooed at the tumor margin to define the primary tumor lesion and in order to assess the response to the neoadjuvant chemotherapy. After tattooing, a cisplatin and 5-fluorouracil combination was intraperitoneally administered as neoadjuvant chemotherapy. Cisplatin (cis-diammine-dichloroplatinum (II)) and 5-fluorouracil (5-fluoro-2,4 [1H,3H]-pyrimidinedione) were diluted with 0.9% saline for injection. The C3H/HeJ mice received 15 mg/kg of 5-fluorouracil on days 1-3 each week and 7.5 mg/kg of cisplatin on day 1 each week. These chemotherapeutic regimens were performed weekly until a response was observed.

Tumor measurement and response assessment. The size of the tumor was measured with sliding calipers three times per week by the same observer and the tumor weight was calculated using the method described by Geran et al. (27). The estimated tumor weight (ETW) was calculated as ETW (mg)=length (mm) x (width (mm))²/2. Additionally, tumor growth was measured as the product of the bidimensional diameters of the tumor mass, at 90º angles from each other (28). A partial response was defined as at least a 50% decrease of the products of the bidimensional diameters without the appearance of new lesions (28). A complete response was defined as the disappearance of all known disease with no new lesions (28). The mice showing a partial response were used in this study.

Tumor resection. The mice were randomly divided into three groups according to the extent of the resection margin at surgery following neoadjuvant chemotherapy. Group A had surgical removal of the tumor with a 5 mm resection margin from the original tattooed tumor lesions although tumor shrinkage had been observed (radical surgery). Group B had surgical removal of the tumor with a 5 mm resection margin from the residual tumor (limited surgery). Group C had surgical removal without a resection margin from the residual tumor so that only the residual tumors were removed (very limited surgery). All the surgical specimens were pathologically reviewed by H&E staining and immunohistochemical staining with cytokeratin. After surgery, all the mice were followed-up until tumor recurrence or death, or for at least 60 days after surgery.

Immunohistochemistry. The formalin-fixed tumor tissues were embedded in paraffin, serially prepared as 5-μm sections and stained, using the avidin-biotin immunoperoxidase method. The

| Table I. The characteristics of mice among the three resection groups*. |
|-----------------|-----------|-----------|-----------|----------|
| Variable        | Group A   | Group B   | Group C   | p-value  |
| N=13            | N=12      | N=12      |           |          |
| Age (weeks)     | 12.8±4.6  | 11.4±4.4  | 11.9±4.9  | 0.74     |
| Before chemotherapy Weight (g) | 25.3±3.8  | 25.8±4.2  | 25.2±4.9  | 0.87     |
| Tumor size (mm²) | 105.5±47.7| 110.9±40.5| 118.7±38.9| 0.73     |
| After chemotherapy Weight loss (%) | 17.8±16.3 | 16.6±14.0 | 19.1±10.8 | 0.72     |
| Tumor size (mm²) | 25.2±13.0 | 23.0±9.9  | 39.2±26.3 | 0.17     |
| Tumor reduction (%) | 75.3±11.7 | 77.8±9.9  | 66.3±13.1 | 0.10     |
| Before operation Weight (g) | 20.5±3.7  | 21.2±3.4  | 24.0±3.3  | 0.79     |

*Mean±S.D. Group A: radical surgery, Group B: limited surgery, Group C: very limited surgery.
sections were deparaffinized, hydrated, placed in citrate buffer (pH 6.0) and heated in a microwave for 20 min. They were then washed and incubated with rabbit polyclonal antimouse cytokeratin (1:50), purchased from Abcam (Cambridge, UK), at room temperature for 1 hour. Secondary antibodies corresponding to the primary antibodies were applied for 30 min at room temperature.

Statistical analysis. The statistical analysis was performed on a personal computer with SPSS 11.0 for windows (SPSS Inc. Chicago, Illinois, USA). Statistical differences of the characteristics of mice were evaluated for the three groups using the Kruskal-Wallis test. The survival rate was calculated by the Kaplan-Meier method and a statistical analysis was performed using the log-rank test. P-values less than 0.05 were considered statistically significant.

Disease-free survival was calculated from the operation until the last follow-up or death from any cause.

Results
Baseline characteristics. Forty mice received neoadjuvant chemotherapy. Three mice had progressive disease. Thirty-seven mice showing a partial response after neoadjuvant chemotherapy received tumor resection. The number and mean age of the mice per group are shown in Table I.

Neoadjuvant chemotherapy and tumor response. The median interval from tumor implantation to the start of neoadjuvant chemotherapy was 11, 13 and 14 days in groups A, B and C, respectively. The weights of the mice and the mean size of the initial tumor mass (the products of the bidimensional diameters) are shown in Table I. The mean tumor weight before the neoadjuvant chemotherapy was 623.4, 660.5 and 739.2 mg in groups A, B and C, respectively (p=0.75).

After the neoadjuvant chemotherapy, the mean tumor weight was 68.9, 58.8 and 139.0 mg in groups A, B and C, respectively (p=0.54) and the rate of tumor reduction by tumor weight was 88%, 90% and 80% in groups A, B and C, respectively (p=0.14). The mean tumor mass and the rate of tumor reduction are shown in Table I.

One or two cycles of chemotherapy were administered. The second cycle was administered to 4 mice in group A, 4 mice in group B and 7 mice in group C (p=0.29).

Surgical outcome. The median interval from the last day of the neoadjuvant chemotherapy to the tumor resection was about 15 days for all the groups. The weights of the mice before tumor resection are shown in Table I.

At surgery, the residual lesions were palpable and no gross residual tumors were left after resection in three groups of mice. All the tumor specimens were reviewed microscopically. Except for a tumor specimen in one mouse of group C, the resection margins for all the tumor specimens were tumor free.

In group A (radical surgery) and group B (limited surgery), no mouse had tumor recurrence after the tumor resection. In group C (very limited surgery), one mouse which had a microscopically positive resection margin

Figure 2. The autopsy finding of one of the mice with progressive disease showing a very infiltrative and locally invasive pattern of the intramuscularly implanted SCC VII tumor.
relapsed on the 19th day and died on the 47th day after the tumor resection. The difference in survival among the three groups was not significant (p=0.64) (Figure 1). Three mice in group A and one in group B died postoperatively due to complications from the resection. In group C, one mouse died postoperatively due to anesthetic complications. By an intent-to-treat analysis, the difference in survival was not significant between groups A and B (p=0.33). Except for the perioperative mortality, the recurrence and survival of limited surgery were similar to those of radical surgery.

Among the mice showing a partial clinical response, three mice in group A, three mice in group B and two mice in group C had pathologically complete remissions. Because of the possibility of failing to identify viable tumor cells in the pathologically confirmed complete remission by H&E staining, all the tumor specimens were reviewed by immuno-histochemical staining with cytokeratin. Among the tumor specimens with a pathologically complete remission by H&E, no viable tumor cells were identified by cytokeratin staining.

The microscopic patterns of the tumor lesions showing partial responses were pathologically evaluated, whether the lesions showing a tumor response had skipped viable tumor cells or not. Except for the residual tumor lesions, viable tumor cells were not observed at the previous tumor sites where a response was shown.

Discussion

An animal model for studying human cancer should reflect the pattern of characteristics of the human cancer. The growth pattern and histological features of SCC VII in head and neck cancer have been previously described. In the murine model of head and neck SCC, extensive local invasion was observed after subcutaneous injection of SCC VII (29, 30). In the current study, tumor tissue was implanted intramuscularly into the buttocks. A feature of the tumor which developed was a very infiltrative and locally invasive pattern that was similar to human head and neck cancer (Figure 2). Therefore, the intramuscular implantation of the SCC VII tumor tissue in C3H/HeJ mice could be used as a model for the evaluation of the resection margins of head and neck cancer.

Some authors have suggested that neoadjuvant chemotherapy acts by cell-killing without a reduction in the extent of disease (7, 31). However, the cytological and stromal changes associated with tumor regression were observed in 77.4% of cases after intraarterial chemotherapy (32, 33). In the present study, with the exception of perioperative mortality, both radical surgery and limited surgery following neoadjuvant chemotherapy resulted in no recurrence. When the tumor lesions showing partial responses from the radical and limited surgery groups were pathologically evaluated, no viable tumor cells or skip metastases were observed at the original tumor sites where the response to chemotherapy was shown. These results suggested that limited or conservative surgery for head and neck cancer might be safe and effective and could be a good treatment option in some subgroups.

The current study had some limitations. Firstly, the model was not orthotropic, the tumor tissue was implanted into the buttock of mice, but SCC VII is an oral squamous cell cancer of CH3/HeJ mice. Secondly, five perioperative mortalities were observed in this study, but they are hardly ever observed in the clinical setting. Thirdly, 60 days might not be enough time to evaluate long-term effects of microscopic residual tumor. An attempt was made to find the optimal surgical technique after neoadjuvant chemotherapy, although it is not easy to standardize surgical procedures and to find a satisfactory study design.

In a meta-analysis of neoadjuvant chemotherapy, a subset analysis of trials of neoadjuvant chemotherapy with cisplatin and 5-fluorouracil compared with locoregional treatment alone showed a survival gain of 5% (25). Given the limited data on neoadjuvant chemotherapy, even though its role continues to be debated, there may be a positive effect with such therapy in certain situations. One consideration is the use of induction chemotherapy to make curative surgery feasible for marginally resectable tumors (34). Reports have shown that platinum-based neoadjuvant chemotherapy could be used in patients with invasive pharyngolaryngeal cancer with intent to cure (21, 22). Based on the above rationale, the feasibility of using neoadjuvant chemotherapy for the conservation of functions associated with the head and neck has been evaluated by the current study.

In the C3H/HeJ SCC VII model, the recurrence and survival of the limited surgery group was similar to those of the radical surgery group after neoadjuvant chemotherapy. If the appropriate subgroup of patients with head and neck cancer could be identified, limited surgery could be an excellent treatment option for patients with head and neck cancer, preserving function without reducing survival. Further studies are needed to define the surgical margin after neoadjuvant chemotherapy in the clinical setting.

Acknowledgements

This study was supported by grants of the Cancer Research Institute, Seoul National University College of Medicine (CRI - 2002 - 9) and from the Innovative Research Institute for Cell Therapy (A062260), Republic of Korea.

References


Received August 12, 2008
Revised December 9, 2008
Accepted December 16, 2008