Expression Level of DNA Topoisomerase Type II Alpha Predicts Chemotherapeutic Effect in Oral Squamous Cell Carcinomas

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DNA topoisomerase type II alpha (Topo IIα) nuclear enzyme is associated with chemosensitivity, chemoresistance and cell proliferation in various neoplastic lesions. This study was performed to clarify whether the Topo IIα/Ki-67 labeling ratio (T/K ratio), which reflects Topo IIα expression level, has significant predictive value in determining response to chemotherapy in oral squamous cell carcinomas (OSCC). We retrospectively reviewed the case of 22 patients with OSCC treated with chemotherapy using a combination of cisplatin and 5-FU (CF therapy). Expression of Topo IIα and Ki-67 was examined by streptavidin-biotin peroxidase using pretreatment biopsy specimens. A significant difference in the mean T/K ratio of the effective group and the ineffective group (P<0.005) was observed. Similar findings were found with regard to Ki-67-LI values (P<0.01). However, the difference was larger in T/K ratios than in Ki-67-LI values. No difference was observed between the chemotherapeutic effects in Topo IIα-LI. The present results suggest that T/K ratios may lead to improved prediction of the effect of CF therapy.

Key words: T/K ratio, Oral squamous cell carcinoma, Immunohistochemistry, Neoadjuvant chemotherapy

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Introduction

A combination of cisplatin (cis-diammine-dichloroplatinum (II); CDDP) and 5-fluorouracil (5-FU) (CF therapy) is an effective chemotherapeutic regimen, with relatively high response rates noted among cases of oral squamous cell carcinoma (OSCC)(1, 2). Use of neoadjuvant chemotherapy is becoming widespread due to the associated advantages, including prevention of distant metastasis, reduction in the size of primary lesions, and reliable surgical excision (3, 4). In clinical practice, however, there are often times when neoadjuvant chemotherapy is insufficient. In such cases, the patient may not receive any benefit in light of the adverse reactions experienced as a result of anticancer therapy. It is therefore necessary to tailor the use of neoadjuvant chemotherapy in individual patients based on predictors of efficacy of treatment.

Since 1984, when Eric et al. (5) discovered that amsacrine (m-AMAS) inhibits DNA topoisomerase II (Topo II), Topo II has been targeted by a number of anticancer drugs (6). Mammalian Topo II is involved in DNA metabolism, including such functions as DNA replication, transcription, recombination, chromosome aggregation and separation of DNA (7). There are two types of Topo II, alpha (Topo IIα; 170 kDa) and beta (Topo IIβ; 180 kDa)(8). It has been reported that Topo II is more sensitive to anti-cancer drugs than Topo IIβ (9). It is known that cancer cells overexpressing Topo II are resistant to CDDP (10, 11), and that expression of Topo II might play an important role in the repair of DNA damage induced by alkylating agents or CDDP (12-14). It can thus be speculated that levels of Topo II expression might correlate with tumor responses to CDDP-based chemotherapy, although these agents do not specifically target Topo II (6).

Recently, an easy immunohistochemical method for evaluating Topo IIα expression was developed in pathology laboratories using an autoclave for antigen retrieval
was defined as 50% or more reduction, no change (NC) 100% disappearance of the tumor, partial response (PR) in various malignant tumors (17, 18). Therefore, the T/K ratio may indicate changes in the expression levels of Topo II. The aim of this study was to determine whether a relationship exists between T/K ratios and the chemotherapeutic effect of CF therapy in OSCC using biopsy specimens. Here we report our preliminary findings regarding the significance of the T/K ratio.

Materials and Methods

Patients and tissues

Twenty-two cases of patients receiving CF therapy for the treatment of primary OSCC, at the Department of Dentistry and Oral Surgery, Jichi Medical School, were selected. All cases underwent an operation after neoadjuvant chemotherapy; 14 were male and 8 were female. Their mean age at the time of operation was 60.9 years (42-78 y). The primary sites of OSCC were tongue (11), mandibular gingiva (5), maxillary gingiva (4), buccal mucosa (1) and floor of the mouth (1).

Biopsy samples from each patient before neoadjuvant chemotherapy were fixed in 10% formalin for 24-48 hours and embedded in paraffin wax. All specimens were reviewed and reclassified as well differentiated, moderately differentiated, or poorly differentiated tumors (19). Of 22 tissue specimens, 14 were well differentiated (described as “high differentiation” tumors), 2 were moderately differentiated, and 6 were poorly differentiated. Moderately and poorly differentiated tumors were then categorized as “low differentiation” tumors.

Chemotherapeutic regimen

All patients were treated with neoadjuvant chemotherapy, also known as CF therapy (1, 2). The chemotherapeutic regimen was a combination of 70 mg/m² CDDP and 96-hour infusion of 5-FU at 700 mg/m²/day. This protocol was defined as one course of treatment.

Assessment of the chemotherapeutic effect

The chemotherapeutic response was clinically evaluated in terms of the percentage reduction in tumor volume on computed tomography (CT) and histological evaluation of the surgical specimens. The chemotherapeutic response was classified according to the general rules for clinical and pathological studies on head and neck cancer (20). Complete response (CR) was defined as 100% disappearance of the tumor, partial response (PR) was defined as 50% or more reduction, no change (NC) was defined as less than 50% reduction, and progressive disease (PD) was defined as an increase in tumor size. Patients with CR and PR were included in the group for whom therapy was effective (effective group), whereas patients with NC and PD were included in the group for whom therapy was ineffective (ineffective group). In the effective group, 4 cases showed CR and 7 cases showed PR. In the ineffective group, all 11 cases demonstrated NC. There were no cases of PD. The CR group received an average of 3.25 courses of therapy, the PR group 3.29 courses, and the NC group an average of 2.27 courses.

Immunohistochemistry

Immunostaining was performed on 3 μm thick serial sections from the same block, which were cut into slices. Immunohistochemical staining for Topo IIa and Ki-67 (MIB-1) antigens was performed using the avidin-biotin complex immunoperoxidase method. After the slides were autoclaved for 5 minutes at 121°C in sodium citrate buffer (2 mM citric acid and 9 mM trisodium citrate dehydrate, pH 6.0), they were kept for 2 hours at room temperature. The slides were incubated in 3% hydrogen peroxide for 5 minutes to block endogenous peroxidase activity and were pretreated for 10 minutes with 10% normal goat serum (Nichirei, Tokyo, Japan). Primary antibodies anti-Topo IIa monoclonal antibody clone 8D2 (dilution of 1:100, provided by Dr. Nozaki, Department of Oral Biochemistry, Kanagawa Dental College, Japan), and anti-Ki67 monoclonal antibody MIB-1 (dilution of 1:50, Immunotech S. A., Marseille, France), were used. The slides were incubated with primary antibodies for 1 hour, followed by exposure to secondary biotinylated antibody (Nichirei) for 10 minutes and exposure to a streptavidin-peroxidase complex reagent (Nichirei) for another 5 minutes. Color was developed with a 0.02% 3,3-diaminobenzidine-tetrahydrochloride solution containing 0.003% hydrogen peroxide in 0.01 M phosphate buffered saline (PBS) for 10 minutes. After washing, the immunostained sections were counterstained in hematoxylin, dehydrated, cleared, and mounted. Sections from a specimen exhibiting follicular hyperplasia of the tonsil served as the positive control. For negative controls, PBS or non-immunized mouse IgG was used instead of primary antibodies.

Measurement of immunohistochemical staining and statistical analysis

For examination of Topo IIa and Ki-67 expression, the same areas were selected in serial sections of the specimens. Using a video micrometer VM-30 (Olympus, Tokyo, Japan), at least 800 cells in random fields (more than five microscopic fields) were examined at x400 magnification. Only strong and weak nuclear staining was regarded as positive, and cytoplasmic staining was regarded as negative. The number of positive and negative cells in

(15). Topo II expression predominantly occurs during the S to G2/M phase of the cell cycle, whereas cells at all stages of the cell cycle, except G0, have been recognized by monoclonal antibody Ki-67 (MIB-1) (16). Topo IIa and Ki-67 are currently the most recognized markers of cell proliferation (16). The ratio of Topo IIa to Ki-67 (T/K ratio) has been implicated in qualitative changes in Topo IIa expression in various malignant tumors (17, 18). Therefore, the T/K ratio may indicate changes in the expression levels of Topo IIa. The aim of this study was to determine whether a relationship exists between T/K ratios and the chemotherapeutic effect of CF therapy in OSCC using biopsy specimens. Here we report our preliminary findings regarding the significance of the T/K ratio.
each specimen was counted by an experienced pathologist (K.T.), who was blinded to clinical information and histological knowledge of the surgical specimens before the cell count. Topo II and Ki-67-positive cell ratios were calculated based on the ratio of immunopositive nuclei to total cells within each specimen. The labeling index (LI) was defined as the average positive cell ratio. Standard deviations for LI values were also calculated. We also determined the Topo II/Ki-67 labeling ratio (T/K ratio) for each sample.

Mann-Whitney U test was employed to examine the correlation between LIs and T/K ratios in each chemotherapeutic effect group. In addition, the relationship between tumor differentiation and chemotherapeutic effect was examined by Fisher's exact probability test. Each test was considered significant when their P-values were less than 0.05.

Results

We detected immunoreactivity for Topo II and Ki-67 in the nuclei of cells on slides from every case. In high differentiation tumors, the expression pattern of Topo II and Ki-67 immunostaining was predominantly localized in the periphery of the cancer nests with cancer pearls. In contrast, more diffuse staining was observed in low differentiation tumors with Topo II and Ki-67 immunolocalization.

The mean Topo II-LI value was 32.1±18.34% in the group for whom therapy was effective (CR+PR), and 34.71±20.94% in the group for whom therapy was ineffective (NC) (Fig. 1A). Mean Topo II-LI values did not differ significantly between the effective and ineffective groups. The mean Ki-67-LI value was 59.09±19.81% in the effective group, and 36.7±14.36% in the ineffective group (Fig. 1B). A significant difference was found among the mean Ki-67-LI values of the two groups (P<0.01, Mann-Whitney U test). The mean T/K ratio was 52.91±21.44% in the effective group, and 90.08±30.07% in the ineffective group (Fig. 1C). The mean T/K ratios were also significantly different among the two groups (P<0.005, Mann-Whitney U test). A greater difference was noted among the mean T/K ratios of the two groups than among the mean Ki-67-LI values. Six patients had T/K ratios greater than 100%, all of whom belonged to the group for whom therapy was ineffective. No one in the group for whom therapy was effective had T/K ratios exceeding 100%. Fig. 2 shows the elevated T/K ratio of a patient for whom therapy was ineffective (121.83). The cancer cells exhibited strong positivity for Topo II and Ki-67.

In addition, of 11 CR+PR cases, high differentiation tumors were observed in six, and low differentiation tumors in five. Of 11 NC cases, high differentiation tumors were observed in nine, and low differentiation tumors in three. No significant correlation was noted between tumor differentiation and chemotherapeutic effect or T/K ratio. The mean LIs of high differentiation tumors were 25.67±12.73% for Topo II and 40.99±12.57% for Ki-67. The mean LIs of low differentiation tumors were 46.99±22% for Topo II and 60.12±20.61% for Ki-67. A significant difference was found between high differentiation and low differentiation in mean Topo II-LI value or mean Ki-67-LI value (P<0.05, Mann-Whitney U test).

No significant findings were found between TNM factors and the T/K ratio or chemotherapeutic effect.
Discussion

A significant difference was noted among the T/K ratios of patients for whom therapy was effective (CR+PR) and those for whom therapy was ineffective (NC) (P<0.005, Mann-Whitney U test). Similar findings were observed with regard to Ki-67-LI values (P<0.01, Mann-Whitney U test). However, a larger difference was noted with T/K ratios than with Ki-67-LI values. In contrast, a significant correlation was not found between Topo II - LI values and chemotherapeutic efficacy. Thus, the T/K ratio was identified as being the most significant indicator of the potential success of therapy, and thus the best way to classify patients for whom therapy might be effective and those for whom it may not. Importantly, patients with T/K ratios greater than 100% showed poor reactivity to neoadjuvant chemotherapy.

With regard to the degree of sensitivity of anti-cancer drugs to Topo II, it has been suggested that quantitative changes in Topo II expression or the production of altered amino acids may lead to qualitative changes in response to therapy (22). The level of cleavable complex formation is also thought to play an important role in this phenomenon (22). The T/K ratio is a ratio of the number of Topo II positive cells, which are detected during the S to G2M phase of the cell cycle, to the number of Ki-67 positive cells, which are detected throughout all phases of the cell cycle (15). Normally, Topo II expression is less than Ki-67 expression in physiological tissue due to its phase-restricted expression which limits the number of cells in tissue which express Topo II at any given point in time (15, 22). However, Topo II is also expressed in the G1 phase of the cell cycle in some cancers, including gastric and ovarian cancer (20, 21). An increased proportion of Topo II positive cells have been observed in malignant tissue, leading to a dramatic increase in the T/K ratio (15, 22). The T/K ratio thus reflects the expression level of Topo II, although increase of T/K ratio reflects the quantitative increase of Topo II positive cells. An elevated T/K ratio has been cited as a key indicator of risk of early relapse of non-Hodgkin’s lymphoma following Topo II - targeted chemotherapy (18). Based on the results of previous studies and the present data, it seems that the T/K ratio is a useful histological parameter by which to predict chemotherapeutic effect.

In addition, a correlation has been observed between Topo II expression level and response to CF therapy based on CDDP, despite the fact that Topo II is not a cellular target of CDDP (6). However, increased levels of Topo II expression have been observed in human cell lines resistant to alkylating drugs or CDDP, and thus, it has been suggested that altered Topo II expression might be important as a mechanism of resistance to alkylating drugs or CDDP (10, 11). Moreover, Eder et al. (23) have implicated Topo II in the repair of DNA damage induced by alkylating drugs. These findings may explain the observed correlation between high T/K ratios and poor tumor sensitivity to CDDP-based chemotherapy.

In summary, calculation of T/K ratios by a patholo-
gist might be useful in predicting the effect of CF therapy. Further examinations of larger numbers of patients are needed to determine the cut off value for the T/K ratio. We hope that this study provides clinicians with useful information regarding the management of OSCC.

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References


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