Pergamon

*Oral Oncol, Eur J Cancer*, Vol. 30B, No. 3, pp. 200–203, 1994

0964–1955/94 $7.00 + 0.00

# Influence of Cisplatinum and 5-Fluorouracil on the Oral Mucosa

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**In a prospective study we examined the effect of preoperative chemotherapy with cisplatinum and 5-fluorouracil (5FU) in 40 patients with a carcinoma of the oral cavity. Histopathological grading, cell kinetic and immuno-histochemical parameters of the mucosa were determined at two different locations. Prior to therapy histological dysplasias "close" to the tumour were observed in the mucosa of 75% of the patients. Dysplastic changes of the mucosa "far" from the tumour were also found in more than one third of the patients. Our results show that more advanced dysplasias improve under the influence of preoperative systemic chemotherapy only temporarily. Moreover, new dysplasias which appeared during chemotherapy and persisted after its termination are probably induced.**

**Keywords: Chemotherapy, oral carcinoma, malignant transformation, second primary tumour.**

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## INTRODUCTION

PROGNOSIS FOR patients with a carcinoma of the oral cavity has not improved significantly during the past 20 years, despite new operative techniques and adjuvant measures like chemo- and radiotherapy [1, 2]. Moreover, increasing numbers of secondary carcinomas are observed, syn- or metachronically manifesting themselves mainly in distant organs, especially the lung and the esophageous [3, 4]. As haematogenous metastases appear relatively late in disease, unfavourable prognosis is rather determined by local recurrences.

Therefore, the goal of our prospective study was to examine the effect of chemotherapy on the oral mucosa.

## MATERIALS AND METHODS

40 patients with carcinoma of the oral cavity were examined. All patients were on schedule for a tumour operation. The planned surgical margin was marked with ink in the mucosa in about 1 cm distance from the clinically visible and palpable tumour. "Tumour close" biopsies were taken from the oral mucosa 1 cm outside this surgical margin (i.e. 2 cm from the tumour). "Tumour far" biopsies were taken from the oral mucosa most distant or opposite to the tumour. The excisions were performed with a scalpel.

All patients received preoperative chemotherapy with cisplatinum (20 mg/m$^2$) and fluorouracil (5FU) (1 000 mg/m$^2$) (over 5 days, repeated at day 22, maximum of three courses) [5]. Two weeks after the last course, radical tumour section was performed using a scalpel for incision. No further therapy, for example irradiation, was applied. Mucosal biopsies were taken directly prior to chemotherapy, at the beginning of each further cycle (i.e. two times), at the time of, and 3 months after the operation.

With all biopsies we carried out a histopathological grading [6]. From this the following classification resulted:

—normal mucosa,
—hyperplastic mucosa,
—dysplasia grade 0 (called "simple leukoplakia" with hyperkeratosis but without nuclear dysplasia),
—dysplasia grade 1–3 (according to the severity of changes).

Further examinations were DNA flow cytometry [7], imaging of the nucleolar organiser region-associated proteins (NORAPs) by the silver NOR staining method and imaging of proliferation marker Ki-67 by the immunohistochemical reaction of the monoclonal antibody Ki-67.

The AgNOR staining was modified according to Ploton *et al.* [8]. Counting of AgNORs was carried out under a light-microscope (Zeiss, Germany) at 2000 × magnification. In each section 100 nuclei were examined for their numbers of nucleoli and AgNORs [9]. Counting was performed in the basal and the first three parabasal cell layers of the mucosa.

The immunohistochemical analysis with monoclonal antibody Ki-67 (dilution 1:100, Dakopatts Comp.) was performed on frozen sections. We used the ABC method (avidin-biotin-peroxidase) [10]. The marker index—defined as the percentage of Ki-67 positive cells—was determined with a light microscope at 400 × magnification. Per section, 20 high-power fields were counted.

The immunohistochemical reaction showing the cytokeratin gene expression was carried out on frozen sections (ABC method [10]). We used:

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Received 19 May 1993; provisionally accepted 26 May 1993; revised manuscript received 4 Sep. 1993.

—monoclonal antibody k 4 (dilution 1:5) (Laboserv, Gießen, Germany)
—monoclonal antibody k 8 (dilution 1:10) (Laboserv)
—monoclonal antibody k 10 (dilution 1:2) (Laboserv)
—monoclonal antibody k 13 (dilution 1:5) (Laboserv)
—monoclonal antibody k 18 (dilution 1:5) (Laboserv)

For statistics we used the Wilcoxon Mann–Whitney rank test.

## RESULTS

Prior to therapy, the mucosa close to the tumour (distance to tumour 2 cm) showed dysplasias at the histological level in 75% of the patients. However, dysplastic mucosal changes were also found far from the tumour in more than one third of the cases (Fig. 1).

Flow cytometry showed that the percentage of cells in S-phase increased with the degree of dysplasia of the mucosa. In patients whose mucosal biopsies revealed high proliferative activity in the first probe (percentage of cells in S-phase > 7.5), this value declined clearly—in the mean—during chemotherapy (Fig. 2a). However, 3 months after the operation a new increase was observed. In contrast, biopsies with a percentage of cells in S-phase smaller than 7.5 in the

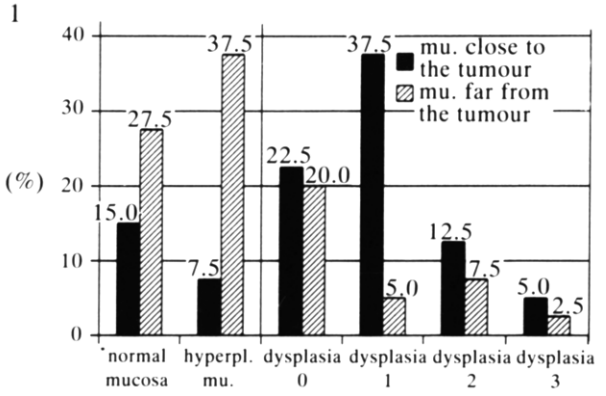


**Fig. 1. Histological grading of the mucosa close to and far from the tumour prior to chemotherapy ($n = 40$). mu, mucosa.**

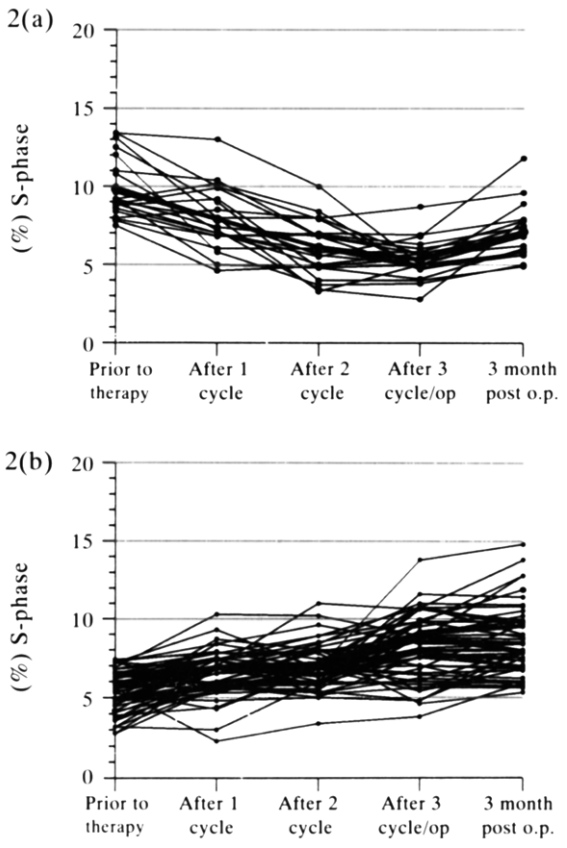


**Fig. 2. (a) Course of the S-phases (%) under chemotherapy with a starting level of greater than 7.5% (n.s.). (b) Course of the S-phases (%) under chemotherapy with a starting level of lower than 7.5% ($P < 0.0001$).**

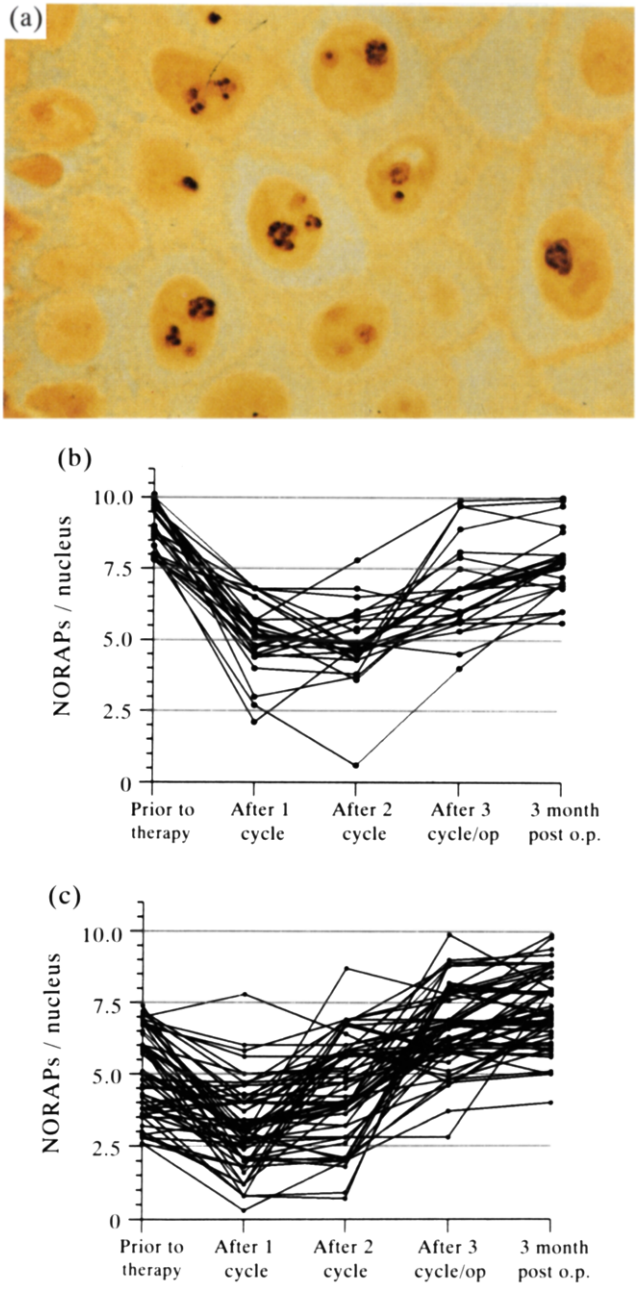


**Fig. 3. (a) Special staining of NOR-associated proteins with silver colloid (AgNORs) in oral epithelia (magnification 1600 ×). (b) Course of the number NOR under chemotherapy for mucosal biopsies with a starting count of greater than 7.5 per cell nucleus (n.s.). (c) Course of the number NOR under chemotherapy for mucosal biopsies with a starting count of less than 7.5 per cell nucleus ($P < 0.00001$).**

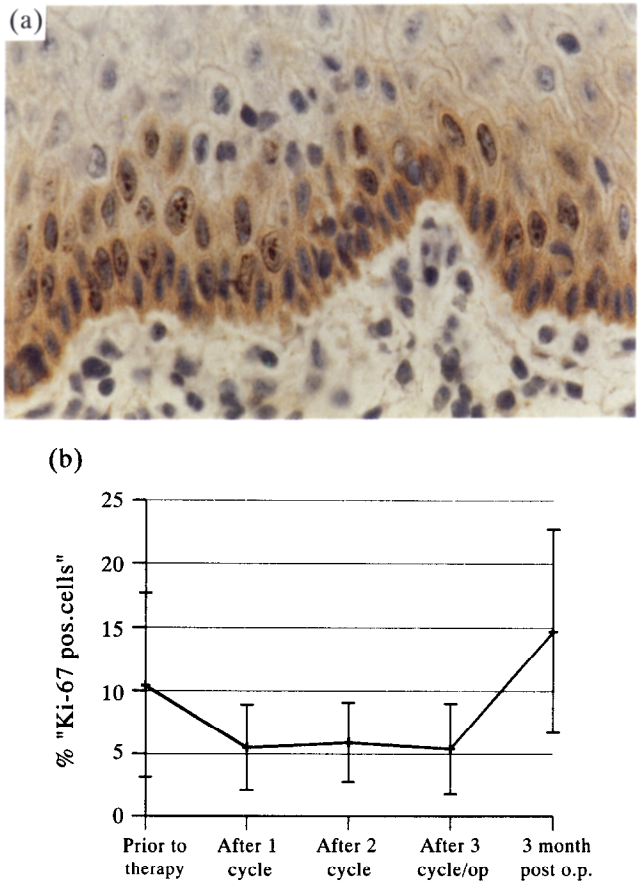


**Fig. 4. (a) Immunohistochemical staining of the oral mucosa with monoclonal antibody Ki-67. Marking of basal and parabasal cell nuclei and partial staining of cytoplasm (ABC method) (magnification 216 ×). (b) Course of the Ki-67 marker index under chemotherapy for mucosal biopsies.**

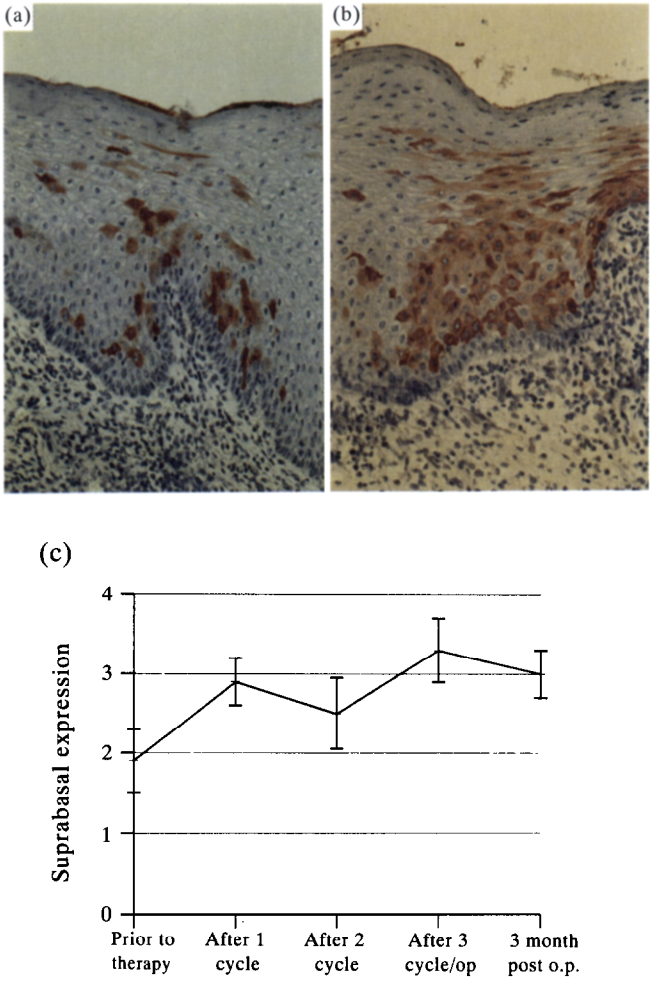


**Fig. 5. (a, b) Imaging of the suprabasal increase of keratin 10 under chemotherapy in epithelial cells (a) prior to therapy, (b) 3 months post-operatively (ABC method) (magnification 108 ×). (c) Expression of keratin 10 at oral epithelial cells under chemotherapy (semiquantitative evaluation).**

beginning, showed an increase in DNA synthesis already 2 weeks after the first cycle was finished (Fig. 2b). Even 3 months after the operation there was no return to the starting level.

Nucleolar organiser regions (NORs) stand for certain DNA areas at five acrocentric chromosomes which are responsible for the ribosomal RNA coding in the nucleolus (Fig. 3a). Number and size of the nucleolus and the NORs change in correlation with the cell cycle. This explains the close correlation between the numbers of NORs and the rate of cells in S-phase [11]. Changes in the quantity of NORs in the mucosa in relation to the starting level show that NOR values starting from both low and high levels clearly drop in the beginning (Fig. 3b, c). Later in therapy, these values rise again, in some cases even above the starting level.

The antihuman monoclonal antibody Ki-67 marks proliferating cells [12]. A correlation between the marker index and the S-phase rate is described [11]. The sections showed small to medium size granules above the nucleus of basal and especially of parabasal cells (Fig. 4a). Under the influence of chemotherapy, the level of the Ki-67 marker index increased subsequent to a temporary drop (Fig. 4b).

As intermediary filaments, cytokeratins, together with actin filaments and microtubuli, construct the cytoskeleton of the epithelial cells [13]. In epithelial cells certain keratins appear by pairs [14]. Not only in carcinomas but also in early dysplastic mucosal changes, a change of cytokeratin gene

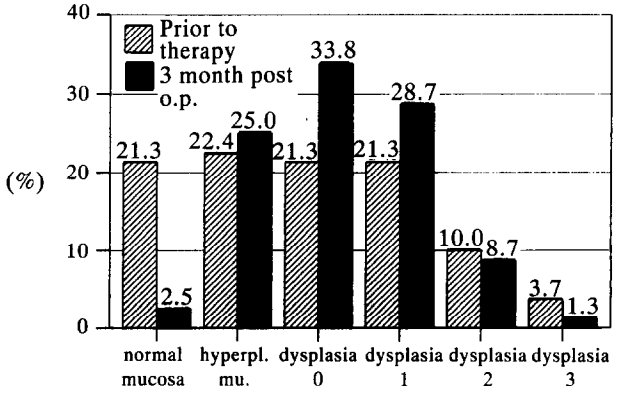


**Fig. 6. Comparison of histopathological findings in the mucosa: prior to therapy and 3 months post-operatively.**

expression (keratin 8 and 18, 4 and 13) is observed. This is especially apparent for keratin 10 which is found suprabasal at keratinisation and with an increasing degree of dysplasia

(Fig. 5a, b). Under chemotherapy there was an intensified expression of this keratin (Fig. 5c).

Comparing the findings prior to chemotherapy with the postoperative histology results, the percentage of biopsies showing a mucosa that was classified as "normal" had declined from 21.3 to only 2.5 (Fig. 6).

## DISCUSSION

In 1953 Slaughter and co-workers [15] defined the phenomenon of multicentricity in patients with a squamous cell cancer of the oral cavity. The authors showed that squamous cell cancer of the oral epithelium appeared to originate from a process of "field cancerisation" in which an area of epithelium was thought to have been preconditioned by still unknown carcinogenic agents. This also led to the concept of a large zone of mucosa at risk, the so-called "condemned mucosa". Our results confirm this hypothesis. Prior to therapy dysplasias at the histological level were found close to the tumour (distance 2 cm) in the mucosa of 75% of the patients. But also, far from the tumour, dysplastic changes of the mucosa were found in more than one third of cases.

In recent years, many drug combinations have been investigated in the treatment of squamous cell carcinoma of the head and neck region. One of the most promising is the combination of cisplatinum and 5FU [16]. The degree of tumour remission after preoperative chemotherapy, however, has no significant influence on the overall survival time [17, 18]. Only a total remission seems to prolong survival time [19, 20]. Even in cases of total tumour remission at the histological level, a "recurrence" can appear after a relatively short interval [21]. Our results show that high-grade dysplasias improve under preoperative systemic chemotherapy only temporarily in most cases. At the same time, new dysplastic regions appear which persist even after termination of chemotherapy. A possible conclusion is that chemotherapy induces an additional malignant transformation in large areas of dysplastically altered mucosa. This may explain the high rate of tumour recurrence despite a high degree of primary tumour remission. On the other hand, patients with oral and pharyngeal cancers have been reported to be at increased risk for second primary tumours, particularly at cancer sites that were linked to tobacco use and alcohol consumption [3, 4, 22]. We think that, apart from its well documented positive effects on the tumour itself, (preoperative) chemotherapy is also to be ascribed carcinogenetic effects that can increase the risk of developing a second malignant tumour.


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