

Phase I Study of Transforming Growth Factor- β Mouthwashes for Prevention of Chemotherapy-induced Mucositis

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ABSTRACT

The purpose of this study was to establish the safety and tolerability of recombinant transforming growth factor- β (TGF- β ; CGP 46614) mouthwashes intended for prevention of chemotherapy-induced mucositis. Local effects were especially analyzed by objective and subjective measurements of mucositis. Secondary aims were analysis of potential systemic exposure and development of anti-TGF- β antibodies. Eleven breast cancer patients received chemotherapy with 1.5 g/m² cyclophosphamide i.v., 80 mg/m² epirubicin i.v., and 1.0 g/m² 5-fluorouracil i.v. ($n = 8$) or 1.6 g/m² carboplatin i.v., 480 mg/m² thiotepa i.v., and 6 g/m² cyclophosphamide i.v. divided over 4 days ($n = 3$). TGF- β mouthwashes (10 ml; provided by Novartis, Basel, Switzerland) were administered for 4 days, four times a day, starting 1 day before chemotherapy. The dose was escalated in following patients from 25 μ g/ml ($n = 3$) to 50 μ g/ml ($n = 3$) and 100 μ g/ml ($n = 5$). Clinically, the mucosa was scored objectively and according to WHO criteria. The percentage of viable oral epithelial cells was determined by trypan blue dye exclusion. Morphology of cells was assessed in buccal smears. Plasma samples were collected for determination of TGF- β levels and anti-TGF- β antibodies. Adverse events were recorded by the patient in a diary. Mouthwashes with TGF- β were well tolerated. Three patients scored for mucositis > grade 0 (WHO grading criteria). The percentage of viable oral epithelial cells in patients treated with 1.5 g/m²

cyclophosphamide i.v., 80 mg/m² epirubicin i.v., and 1.0 g/m² 5-fluorouracil i.v. was stable, whereas in patients treated with 1.6 g/m² carboplatin i.v., 480 mg/m² thiotepa i.v., and 6 g/m² cyclophosphamide i.v. divided over 4 days, an increase was observed. The morphology of buccal cells showed a transient shift from mature to immature cells in the first week. Neither systemic absorption of TGF- β nor development of TGF- β antibodies was observed. TGF- β mouthwashes were well tolerated and deserve further study in preventing chemotherapy-induced mucositis.

INTRODUCTION

Mucositis is increasingly emerging as a dose-limiting toxicity in the treatment of cancer patients with high-dose chemotherapy, especially because hematological growth factors and the reinfusion of peripheral stem cells have become ubiquitous in such treatments (1). The faster recovery of bone marrow allows multiple transplantations with higher cumulative dosages of chemotherapy, causing novel patterns of toxicity (2). Chemotherapy induces mucositis by its direct toxic effects on the rapidly dividing basal epithelial cells of the oral mucosa. The damaged mucosa is very susceptible to secondary infection, which, in turn, can lead to septicemia in neutropenic patients. Moreover, oral mucositis impairs quality of life because of pain and interference with oral intake (3). Prevention of mucositis, therefore, is an important goal of supportive therapy. In the past, many intervention studies have been performed, with marginal success, with various agents such as allopurinol (4), sucalfate (5), mesalazine (6), pentoxifylline (7), glutamine (8-10), mistoprolol (11), interleukin 11 (12), and GM-CSF² (13), by systemic administration as well as topical application (14, 15).

Recently, new insights have emerged concerning the possible mechanisms of mucositis, and these, combined with the development of recombinant homologues of the naturally occurring TGF- β , gave the opportunity to study a genuine preventive strategy for chemotherapy-induced mucositis (16, 17).

TGF- β exists in three isoforms, which exert inhibitory effects on the proliferation of several cell types by inducing a reversible arrest of cells in G₁ (18-21). TGF- β protected epithelial cells *in vitro* from damage caused by several cytotoxic drugs acting in the S or M phase of the cell cycle (16, 21). *In vivo* experiments with TGF- β , applied locally or injected s.c. in hamster buccal epithelium, showed reduced proliferation of the basal oral epithelium layer as well as a reduction in the incidence, severity, and duration of 5-fluorouracil-induced mucosi-

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² The abbreviations used are: GM-CSF, granulocyte macrophage colony-stimulating factor; TGF- β , transforming growth factor- β ; CEF, 1.5 g/m² cyclophosphamide i.v., 80 mg/m² epirubicin i.v., and 1 g/m² 5-fluorouracil i.v.; CTC, 1.6 g/m² carboplatin i.v., 480 mg/m² thiotepa i.v., and 6 g/m² cyclophosphamide i.v.

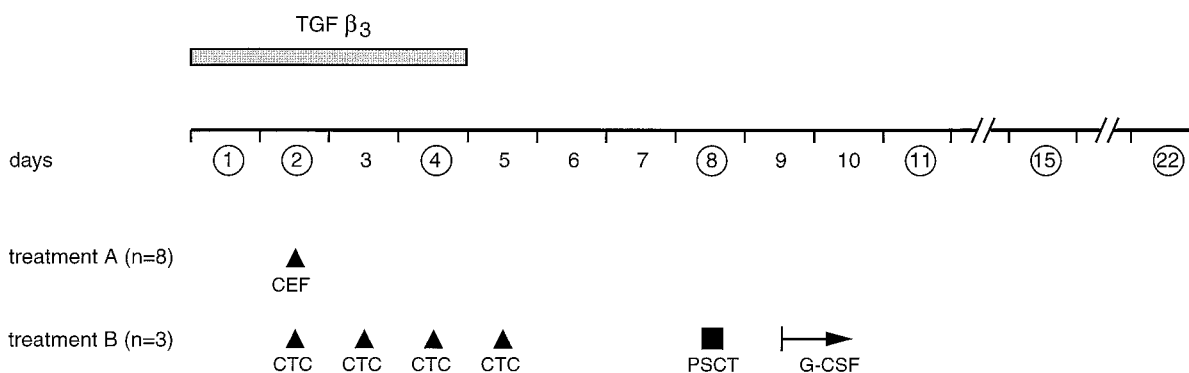


Fig. 1 Treatment schedule of TGF-β mouthwash four times a day during chemotherapy. Circled numbers, days on which oral mucosa was evaluated, clinically and cellularly. PSCT, peripheral stem cell transplantation (autologous; cryopreserved); G-CSF, granulocyte colony-stimulating factor.

tis (16, 17). The TGF-β3 dose used for local application in these animal studies varied between four doses of 20 μg over 24 h for the mucositis model and five doses of 200 μg over 48 h for the cell cycling model, which was developed for measurement of TGF-β3-induced changes in oral epithelial cell cycling. In this latter model, the submucosal injections consisted of five doses of 20 μg in 48 h. In view of these promising results in animals, a Phase I study was performed on the safety, tolerability, and potential systemic side effects of locally applied recombinant TGF-β3 (CGP 46614) in patients scheduled to receive intermediate stomatotoxic chemotherapy. Because of the Phase I character of the study, no high-dose stomatotoxic chemotherapy was used. The secondary aims were assessment of severity and duration of oral mucositis and analysis of systemic drug exposure as well as determination of the possible development of anti-TGF-β3 antibodies in patients. In addition to common safety assessments and clinical mucositis scoring systems, effects of treatment with TGF-β3 were evaluated by an objective *in vitro* assay, in which the percentage of viable oral epithelial cells was determined and the morphology of buccal epithelial cells was assessed (22).

PATIENTS AND METHODS

Patients. Patients with histologically proven metastasized or locally advanced breast cancer who were scheduled to receive intermediate to high-dose chemotherapy could be included after giving informed consent. Patients with ulcerative lesions in the oral cavity or any grade of mucositis in the last 4 days before study onset were excluded, as were patients with Sjögren's syndrome. Also excluded were patients who were pregnant or lactating or who did not use effective means of contraception; patients with other malignancies, renal dysfunction, or hematological disorders; patients who had been treated with corticosteroids, topical antibiotics, or disinfectants within the 2 weeks prior to study entry; or patients who had been treated with other investigational drugs within the 4 weeks prior to study entry. Smoking habits and alcohol consumption were recorded on all patients because of the potential effects on oral mucosa. The study was approved by the local medical ethical committee, and all patients gave written informed consent.

Treatment. Chemotherapy was administered within two protocols (see below), the treatment schedules are presented in Fig. 1. Chemotherapy-naïve patients with metastasized breast cancer received treatment schedule A: CEF on day 2, repeated every 3 weeks. Patients with locally advanced breast carcinoma with at least four positive axillary lymph nodes received treatment schedule B: CTC divided over days 2–5, which was followed by peripheral stem cell reinfusion on day 8 (23). The patients in schedule B were pretreated with induction chemotherapy with four cycles of 500 mg/m² 5-fluorouracil i.v., 90 mg/m² epirubicin i.v., and 500 mg/m² cyclophosphamide i.v. every 3 weeks. The last course of 500 mg/m² 5-fluorouracil i.v., 90 mg/m² epirubicin i.v., and 500 mg/m² cyclophosphamide i.v. was given at least 4 weeks before start of CTC. Before treatment, a comprehensive oral and dental evaluation was performed, including radiographic examination. All potential risk factors and foci for oral complications during the neutropenic phase were eliminated appropriately, if required. In all CTC patients, recombinant granulocyte colony-stimulating factor (Amgen, Thousand Oaks, CA) was started on day 8 until leukocyte recovery to $>3.0 \times 10^9$ cells/liter.

CGP 46614, recombinant protein homologue of TGF-β3 (provided by Novartis, Basel, Switzerland) was administered as a 10-ml dose of mouthwash because this volume allows uniform distribution to all regions of the oral cavity. The mouthwash was used four times a day in the morning, midday, evening, and before bedtime over 4 days, starting 1 day before chemotherapy. Before swallowing, patients rinsed and gargled the mouthwash for 1 min. Afterward, they were not allowed to eat, drink, or rinse with other mouthwashes for 1 h. The dose range and schedule of TGF-β3 were based on animal data, in which hamsters received between four doses of 20 μg over 24 h and five doses of 200 μg over 48 h (16, 17). The dose level for the first three patients was 25 μg/ml; the following dose step (50 μg/ml) was administered to three patients, and five patients received the maximal dose of 100 μg/ml, which appeared to be the maximal feasible dose with respect to pharmaceutical manufacturing. Implementation of lower doses, to a minimum of 2.5 μg/ml, which was foreseen in the case of toxicity, was not necessary. Patients on treatment schedule A who were outpa-

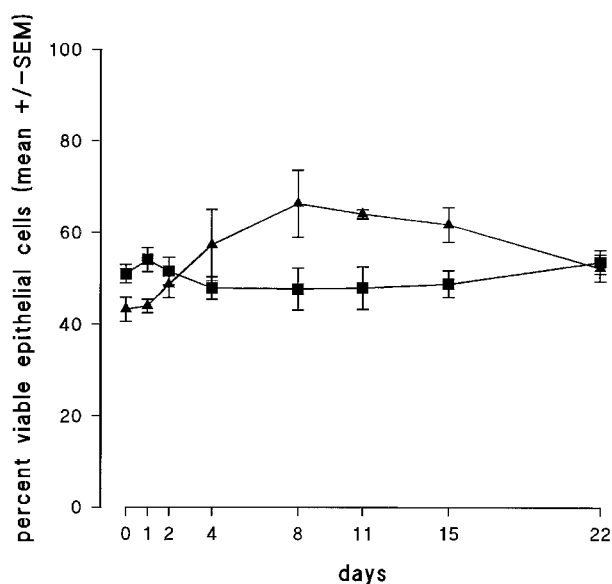


Fig. 2 The increase in percentage viable epithelial cells after chemotherapy and TGF- β 3 mouthwash is more readily seen after the CTC regimen (▲) than after the CEF regimen (■).

tients were trained to reconstitute the dry TGF- β 3 powder vials with solvent that had to be diluted with an equal amount of water (aqua for injections provided in ampules). For the hospitalized patients from treatment schedule B, the reconstitution procedure was performed at the hospital pharmacy. The reconstituted colorless and tasteless viscous solution was used immediately. Each patient was treated with TGF- β 3 for only one chemotherapy cycle.

Measurements. Measurements were performed by a trained physician on day 1 before treatment with TGF- β 3, day 2 (start of chemotherapy), day 4, day 8, day 11, day 15, and day 22. The oral cavity was inspected for oral hygiene, gingival bleeding, and xerostomia. In addition, an objective mucositis score was assessed, in which the oral cavity was divided in seven regions: buccal mucosa; sulci; floor of mouth, lingual frenulum, and ventral tongue; borders of tongue; labial mucosa; pterygomandibular raphe and soft palate; and attached gingiva and hard palate. The left and right side seven regions were separately scored for erythema and number of ulcerations. Also, a score was assessed, according to the WHO toxicity grading criteria as follows: grade 0, normal/no mucositis; grade 1, soreness and erythema; grade 2, erythema and ulcers/patients can eat solids; grade 3, ulcers/patient requires liquid diet only; and grade 4, alimentation not possible (24).

At all visits, the percentage of viable oral epithelial cells in an oral washing was determined, the methods for this assay have been described previously (22). On the same days, a smear with a cytobrush of the buccal mucosa was taken and spread on microscope slides. This buccal smear was stained according to Papanicolaou, which was followed by assessment of epithelial cell morphology and differentiation/maturation (22). The orange, irregular shaped, sometimes flattened cells were classified as mature, whereas the blue/green, smaller, rounded cells were

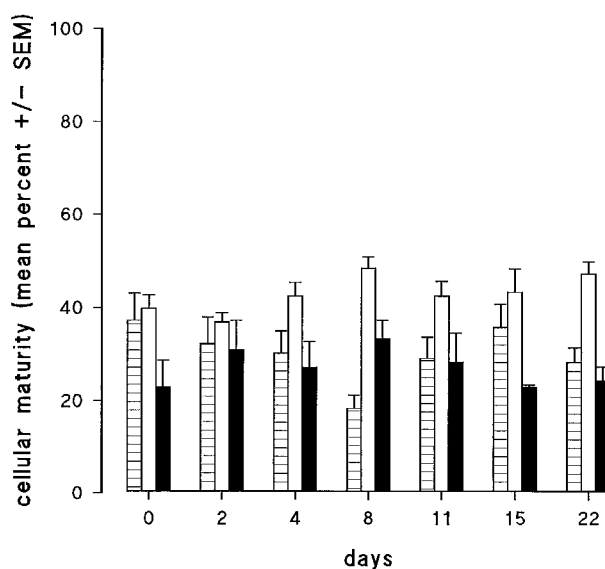


Fig. 3 Types of buccal mucosal cells after chemotherapy and TGF- β 3 mouthwash. ▨, mature; □, intermediate; ■, immature.

categorized as immature cells. Cells with a partly orange and partly green appearance were graded as intermediate cells. On each smear the percentages of mature, intermediate, and immature cells as well as the ratio of immature:mature cells were determined. After logarithmic transformation, this ratio was compared with previously obtained data of buccal cell-morphology of 12 patients treated with identical chemotherapy with CTC without TGF- β 3 (22).

Plasma samples for determination of TGF- β 3 levels were collected on days 1 and 3 or 4 before as well as 0.5, 1, 2, and 4 h after administration of TGF- β 3. In addition, at baseline and at day 22, serum was analyzed for the presence of anti-TGF- β 3 antibodies. Both TGF- β 3 levels (determination limit, 10 pg/ml) and antibodies were determined by ELISA methods at Bioanalytics and Pharmacokinetics, Pharma Research and Development, Novartis, Basel, Switzerland (25, 26).

In diaries, patients registered complaints as well as intensity and duration of oral pain and pain on swallowing by means of a visual analogue scale, in which 0 represents no pain and 10 represents extreme pain. Concomitant medications, including analgesics, as well as adverse events were also registered in the diaries. Laboratory evaluations consisted of blood counts and renal and liver function analyses. All side effects were recorded and scored according to the WHO toxicity grading scale (24).

Statistics. Statistical analysis was performed by comparing data by parametrical and nonparametrical analysis, when appropriate. $P \leq 0.05$ was considered to be significant.

RESULTS

Between April 1996 and April 1997, 11 patients were included, 8 with metastasized (treatment schedule A) and 3 with locally advanced breast cancer (treatment schedule B). The age of the patients ranged from 29 to 52 years. One patient was a

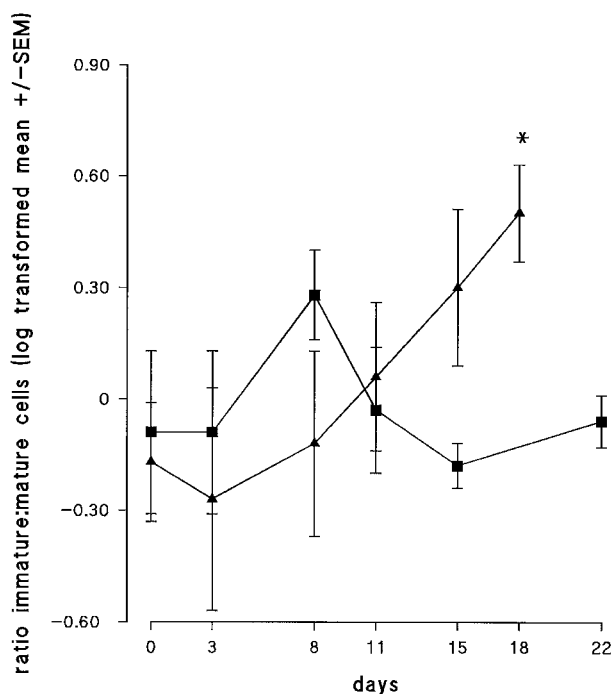


Fig. 4 Ratio of immature to mature cells in patients after chemotherapy and TGF- β 3 mouthwash peaks earlier than that in patients treated with chemotherapy alone. ■, TGF- β 3 mouthwash; ▲, historical control group, *i.e.*, no TGF- β 3. *, significant.

smoker (five cigarettes a day) and was included in the highest TGF- β 3 dose group; three patients had previously smoked but had refrained from smoking for 1, 6, and 15 years; and the remaining patients had never smoked. Eight patients were moderate alcohol consumers (maximum of 2 drinks per week), and the other three patients did not drink alcoholic beverages. Of the eight patients with metastasized breast cancer, one was treated with TGF- β 3 in the first course of chemotherapy, six were treated in the second course, and one was treated in the third course of CEF chemotherapy. One of the eight patients who received prior CEF chemotherapy (without TGF- β 3) did demonstrate mucositis during one of these courses of chemotherapy (grade 1 WHO). In addition, one of the three patients with locally advanced breast cancer experienced mucositis during induction chemotherapy (grade I WHO). The oral and dental evaluation before chemotherapy revealed disorders in three patients; two required tooth extractions. In the third patient, a gingival pocket was treated. Because of the small numbers, due to the Phase I character of the study, no comparisons between the three dose groups of TGF- β 3 could be performed as far as safety evaluations and mucositis measurements were concerned. The inspection of the oral cavity for assessment of oral hygiene, gingival bleeding, and xerostomia revealed local plaque or debris at least at one visit in five patients, and three of them had complaints of dry mouth at one or more visits. No gingival bleeding was observed. The objective oral mucositis score revealed disorders in five patients: one patient experienced erythema at two sites, two patients experienced a single lesion at one site, and two patients experienced erythema as well as

lesions at multiple sites. A mucositis score of grade >0 according to WHO scoring criteria was recorded for three patients (two CEF; one CTC), with a maximum of grade 3 scored by one patient, which seemed at least partially related to a tooth extraction performed on day 1.

None of the patients recorded pain on the visual analogue scale during the 4 days of TGF- β 3 treatment, but three patients recorded pain of grade >0 afterward and consequently received analgesics for mucositis. The patient with the mucositis score of WHO grade 3, who had received TGF- β 3 at a dose level of 25 μ g/ml and CEF chemotherapy, developed increasing pain on day 8 and used nonmorphine analgesics (acetaminophen and tramadol hydrochloride) for 6 days starting on day 9. The remaining two patients with pain scored mucositis grade 0 according to WHO criteria and received CTC chemotherapy and TGF- β 3 at a dose level of 100 μ g/liter. They used morphine for 1 (day 16) and 2 days (days 15 and 16) for pain localized in the throat and esophagus, respectively, and these sites were not assessable for inspection.

Fig. 2 shows the mean percentage of viable oral epithelial cells during study. The initial mean percentage \pm SE of viable oral epithelial cells was $49 \pm 2\%$, and rose to $53 \pm 5\%$ on days 8 and 11 (not significant). However, subgroup analysis showed in patients treated with the more intensive bone marrow ablative CTC regimen, a significant increase from the mean baseline value of $43 \pm 3\%$ to a level of $66 \pm 7\%$ ($P \leq 0.05$) at day 8. In patients treated with CEF, this percentage remained unchanged.

The morphology of Pap-stained buccal epithelial cells during study with TGF- β 3 (all patients) is shown in Fig. 3. The percentage of mature cells varied between 37 ± 3 (day 1) and 18 ± 3 (day 8), the percentage immature cells varied between 23 ± 6 (day 1) and 33 ± 4 (day 8), and the percentage of intermediate cells 37 ± 2 (day 2) and 48 ± 2 (day 8). No differences were found between both treatment groups. The ratio of immature:mature cells, shown in Fig. 4, did show a significant increase at day 8 ($P < 0.05$). The ratio 2 and 3 weeks after the start of chemotherapy was not different from the ratio before chemotherapy. Both treatment groups showed the same general pattern. This pattern contrasts with that found previously in 12 CTC-treated patients without TGF- β 3, where the ratio still is enhanced at day 17. This day 17 ratio is higher than the day 14 ratio ($P < 0.01$) and day 21 ratio ($P < 0.005$) in TGF- β 3-treated patients.

In one patient, plasma TGF- β 3 levels between 45 and 73 pg/ml were detected before exposure to TGF- β 3 as well as consistently during treatment; in the other patients, no TGF- β 3 was detected in plasma. In addition, no anti-TGF- β 3-antibodies were detected in serum samples of the patients.

The most frequently observed side effect was nausea in six patients (CEF, $n = 3$; CTC, $n = 3$), starting at day 4 ($n = 5$) or day 5 ($n = 1$); this was most likely attributed to chemotherapy. Laboratory test showed low leukocyte counts ($<3.0 \times 10^9$ cells/liter) in all patients after chemotherapy and low platelet counts ($<100 \times 10^9$ cells/liter) in three patients treated with CEF and in all patients treated with CTC. All CTC-treated patients required platelet as well as RBC transfusions. In one patient, hepatic dysfunction and a rash, which were regarded as an allergic reaction to antibiotic treatment, were observed. After

antibiotics were changed, elevated liver functions gradually improved. This patient did not experience mucositis.

DISCUSSION

Mucositis is an important side effect following treatment with high-dose chemotherapy because of the coinciding susceptibility to secondary infections and septicemia. Moreover, it is highly detrimental to the quality of life for sometimes weeks after such treatments. Hence, an effective preventive treatment could be of great value. Recent studies with the recombinant TGF- β 3 showed prevention of 5-fluorouracil-induced mucositis in animals (16, 17). Therefore, this Phase I dose-escalating study was performed to evaluate the tolerability, safety, and possible antibody development of mouthwashes with TGF- β 3 in patients treated with mildly stomatotoxic chemotherapy. The maximal dose in this study (100 μ g/ml; cumulative dose, 16 mg) considerably exceeds the maximal topical applied dose used in animals (cumulative dose, 1 mg). Preventive effects were observed in those animals at much lower dose levels (cumulative dose, 80 μ g; Ref. 17). Therefore, no attempts have been made to reach a maximal tolerable dose in this Phase I study. In addition, the dose level of 100 μ g/ml appeared the maximal feasible dose with respect of pharmaceutical manufacturing. Because of the Phase I character of the study, no extreme stomatotoxic chemotherapeutic regimens were used to get an impression of the local tolerability of the mouthwashes.

Although the number of patients was limited, the drug seems to be safe because no unexpected events were observed, and none of the recorded adverse events were attributed to TGF- β 3. Nausea, the leading adverse event, was ascribed to chemotherapy, which was supported by the fact that nausea was reported between days 4 and 19, whereas at visit 2, before chemotherapy and after start with TGF- β 3 treatment, no nausea was experienced.

It seems that TGF- β 3 mouthwashes do not lead to systemic absorption of TGF- β 3 because, in all but one patient, no TGF- β 3 could be detected in the plasma. The low and constant levels of TGF- β 3 detected even before exposure with TGF- β 3 in this single patient suggest either production of endogenous TGF- β or of a compound cross-reacting in the ELISA. *In vitro*, TGF- β 3 has shown blocking effects on human bone marrow and hematopoietic progenitor cells (18). Sonis *et al.* (17) observed in hamsters no effects of locally applied TGF- β 3 on leukocyte or platelet counts, whereas submucosal injected TGF- β 3 resulted in a dose-dependent reduction of leukocytes. Other examples of absorption of locally applied proteins have been reported. In mice, p.o. administered antidoxorubicin monoclonal antibodies penetrated into the epithelium and muscularis mucosa of the small intestine and reduced doxorubicin-related apoptosis in intestinal crypts with a 0.5% absorption of protein associated radioactivity in the blood (27). After mouthwashes with recombinant GM-CSF, 5 of 34 patients showed detectable levels of GM-CSF in plasma (14). Clinically, the incidence, severity and duration of mucositis observed in the study was as expected in this patient groups. In patients treated with CEF, mucositis was comparable with mucositis noted after preceding courses of CEF chemotherapy without TGF- β 3. Mucositis in treatment

schedule B was previously reported as mild because only 3–21% of patients experienced grade III mucositis according to the WHO (2, 28).

Because this study was a Phase I study, designed for establishing safety and tolerability of TGF- β 3 mouthwashes, special attention was given to possible local effects of TGF- β 3 either indicating amelioration or aggravation of mucositis. For this purpose, in addition to the ordinarily used clinical mucositis scoring systems, such as the WHO system, we applied a recently developed, objective, *in vitro* assay of chemotherapy-induced mucositis. This assay was developed because all available scoring systems are descriptive, based on either general appearance of the oral cavity or a combination of changes in the oral mucosa with subjective complaints and functional impairment, thus making comparisons between different scoring systems difficult (22, 29).

In CEF-treated patients, only a small insignificant rise in percentage of viable oral epithelial cells was observed, whereas in the CTC-treated patients, a previously described significant rise was confirmed (22). An explanation for this difference could be that CEF is a less toxic regimen with respect to oral mucosa than CTC. Another reason could be the discrepancy in the time elapsed after previous chemotherapy, which was 20 days for CEF-treated patients compared to 30 days for CTC-treated patients. This dissimilarity probably accounts for both the difference in the baseline percentage of viable oral epithelial cells and the vulnerability of the mucosa for current chemotherapy.

The morphology of Pap-stained oral epithelial cells showed, in a previous study without TGF- β 3, a progressive shift from mature to immature cells over the entire 3 weeks, whereas in this study, only a transient rise after 1 week was observed (22). This could suggest a TGF- β 3-mediated reduction of vulnerability of the mucosa. This is in accordance with results of Sonis *et al.* (17), who observed, after topical application of TGF- β 3 to hamster buccal mucosa, a reduced basal cell proliferation measured by proliferation cell nuclear antigen immunohistochemistry and DNA ploidy.

The formerly most important side effect of high-dose chemotherapy, myelosuppression, gradually declines as dose-limiting toxicity because of the expansion of hematological supportive care methods such as peripheral blood stem cell reinfusion and use of several hematological growth factors. Consequently, extramedullary toxicities emerge as dose limiting for further dose escalation of cytotoxic chemotherapy. Therefore, new supportive care methods are required. Preferably, sophisticated drugs should be used to prevent particular organ toxicity in this context. Locally applied TGF- β 3 could be one such drug, in view of its safety, tolerability, and lack of a tendency to provoke antibody development. Therefore, further Phase II studies with TGF- β 3 mouthwashes (dose level, 100 μ g/ml) intended for prevention of chemotherapy-induced mucositis are justified.

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