

# Efficacy of granulocyte macrophage colony-stimulating factor on oral mucositis

Granülosit-makrofaj-koloni uyarıcı faktörün oral mukozit tedavisinde etkinliği

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## OBJECTIVES

Mucositis is a common toxicity in head and neck cancer irradiation, and can cause dose-limiting in patients. There is no widely accepted effective treatment or prevention. The purpose of this study was to investigate the efficacy of granulocyte macrophage colony-stimulating factor (GM-CSF) as a mouthwash to prevent radiation therapy (RT)-induced oral mucositis.

## METHODS

Thirty-two head and neck cancer patients were enrolled in the study and evaluated prospectively. Lesions were scored using the Radiation Therapy Oncology Group (RTOG) criteria. Variables were age, sex, history of smoking, anatomic region, cancer stage, radiation area, and applied surface area.

## RESULTS

Grade III-IV mucositis developed in 22 patients (68%) during RT. The only statistically significant relation was between the presence of mucositis and a history of smoking ( $p=0.04$ , chi-square). Topical GM-CSF (400 µg 250 cc/day) application had no effect on 1 patient (4%), while 14 patients (64%) showed some improvement and 7 patients (32%) had complete healing. The results of subjective and objective scores were well correlated. GM-CSF had no effect on oral flora, and there was no change in peripheral neutrophil counts.

## CONCLUSION

Topical use of GM-CSF shows promising effects in controlling RT-induced oral mucositis.

**Key words:** Cytokine; GM-CSF; mucositis; radiotherapy; toxicity.

## AMAÇ

Baş-boyun kanserlerinde RT'ye bağlı mukozit gelişimi önemli bir doz sınırlayıcı yan etkidir. Özellikle oral mukozayı koruyarak tümöre etkin dozun verilmesi sıklıkla mümkün olamamaktadır. Çalışmamızda radyoterapiye (RT) bağlı oral mukozitin tedavisinde ağız içi çalkalama solüsyonu olarak granülosit-makrofaj-koloni uyarıcı faktör (GM-CSF) uygulamasının etkinliği araştırıldı.

## GEREÇ VE YÖNTEM

Baş-boyun kanseri nedeniyle 32 hasta çalışmaya alındı ve sonuçlar prospektif olarak incelendi. RT sırasında gelişen oral lezyonlar RTOG ölçütlerine göre skorlandı. Mukozit ve tedavi sonu cevap yaş, cinsiyet, sigara anamnezi, kanser bölgesi, evre ve RT alanlarına göre değişiklikleri ayrıca değerlendirildi.

## BULGULAR

RT sırasında grad III-IV mukozit 22 hastada (%68) gelişti. Mukozit gelişimi üzerinde istatistiksel olarak anlamlı olan tek faktör sigara anamnezi olarak bulundu ( $p=0.04$ ). Topikal GM-CSF uygulaması (400 µg 250 cc/gün) 14 hastada (%64) mukozit tedavisinde etkili oldu, 7 hastada (%32) lezyonlarda tam yanıt alındı. Bir hastada (%4) tedaviye yanıt alınmadı. Subjektif ve objektif yanıt değerlendirmeleri birbiriyle uyumlu bulundu. Periferel nötrofil sayısı veya oral flora üzerinde değişiklik görülmedi.

## SONUÇ

Topikal GM-CSF uygulaması RT'ye bağlı mukozitin tedavisinde ümit vaat eden bir çözüm olarak görülmektedir.

**Anahtar sözcükler:** Sitokin; GM-CSF; mukozit; radyoterapi; toksisite.

Development of hemotoxicity and mucositis are well-known side effects of chemotherapy (CT) and/or radiotherapy (RT) used to cure head and neck cancers.<sup>[1,2]</sup> While hematotoxicity can be successfully controlled by various agents, mucositis is still a major limiting factor.<sup>[2]</sup> Difficulties in nutrient uptake, severe pain and secondary infections associated with mucositis cause temporary withdrawal of RT.<sup>[1,3]</sup> Several chemotherapeutic agents including lidocaine, dyclonine and cytokines have been in trial to control mucositis during CT and/or RT.<sup>[4,5]</sup> Agents that modify salivary flow rate or antibacterials that target oral flora have also been used.<sup>[6]</sup>

Hematopoietic growth factors have proven efficacy in reducing certain toxicities induced by various chemotherapeutic agents.<sup>[7]</sup> The colony-stimulating factors, granulocyte or granulocyte-macrophage (G-CSF, GM-CSF), stimulate proliferation and maturation of myeloid progenitors and have been effective in reducing neutropenia and its complications.<sup>[7]</sup> The use of CSFs may also reduce the incidence and severity of mucositis.<sup>[7,8]</sup> Some authors suggest that GM-CSF, which is systemically used to control hemotoxicity, can also be an alternative to control oral mucositis when used locally.<sup>[9-12]</sup>

The purpose of this study was to determine prospectively the effect of GM-CSF as an oral rinse to control Grade III-IV (G III-IV) oral mucositis development during RT.

## MATERIALS AND METHODS

Thirty-two patients who were diagnosed with head and neck cancer and were scheduled for RT treatment from July 1999 to May 2000 at the Institute of Oncology, Istanbul University were included in the study. The Istanbul University Oncology Institute Board approved the study.

### Patient Characteristics

According to Karnofsky Performance Test, patients should have scores of 70 and above. Median age was 53 years (range: 13 to 74 years) and the male/female ratio was 14/18 (Table 1). The most commonly diagnosed cancer type was nasopharyngeal carcinoma (10 patients), followed by cancer of the oral cavity (6 patients), larynx (5 patients),

tongue (5 patients), maxillary sinus (4 patients), and parotid gland (2 patients). Four patients were stage II; 18 patients stage III and 10 patients stage IV. Twelve patients were smokers. None of these patients continued to smoke during RT. Eighteen cases were postoperative and 14 cases had biopsy only taken before RT (Table 1). Informed consent for the use of topical GM-CSF was obtained from each patient before RT.

### Oral Health Examination

Screening for oral health problems was completed by a dentist at the beginning of the therapy. Any diagnosed infection source was eliminated and teeth with guarded or poor prognosis were extracted. Intraoral microbiological examination was performed at the beginning and during RT when G I-II mucositis developed and was repeated when G III-IV mucositis was present (Table 2). Scraped material in Sprout medium was sent to the laboratory. Cultures were incubated within blood agar and dextrose agar at 37°C for 48 hours in an anaerobic condition, and bacterial proliferation was assessed. Only one patient was diagnosed with oral candidiasis and treated before RT.

**Table 1**  
Patient demographics and the factors affecting mucosal reactions

	Mucosal toxicity		p
	Grade III-V	Grade I-II	
Gender			
Female	9	5	0.92
Male	13	5	
Stage			
II	3	1	1.0
III	12	6	
IV	7	3	
Smoking history			
Positive	11	1	0.04
Negative	11	9	
Surgical intervention			
Positive	12	6	0.77
Negative	10	4	
RT surface area (of total oral mucosa)			
>1/2	25	5	0.55
<1/2	7	5	

**Table 2**  
Scoring in oral mucositis

	I	II	III	IV
Objective (gross)	Diffuse erythema	Erythema, small foci of ulcers	Ulcers covered by pseudo-membranes in <1/2 of mucosa	Necrotic ulcers and hemorrhage
Subjective (functional)	Mild soreness, solid diet	Mild to moderate pain, soft diet	Severe pain, dysphagia, liquids only	Severe pain, liquids only and/or parenteral support

### Hematological and Biochemical Tests

Routine tests were performed at the beginning and during RT once a week to monitor hemoglobin, hematocrit, leukocyte, neutrophil and platelet levels, blood urea nitrogen (BUN), creatinine, alkaline phosphatase, and liver enzymes.

### Radiation Therapy

All patients were treated using a Co<sup>60</sup> machine and parallel opposite fields. RT was received in 23 patients with conventional fractionation 50-70 Gy, 200 cGy/fr. In 9 patients with nasopharyngeal carcinoma, RT was started with 180 cGy/fr and during the last two weeks, accelerated fractionation with twice daily RT was applied. The RT field included the tumor and/or all lymphatic areas depending on the specific cases.

### Chemotherapy

In patients having accelerated fractionation, three cures of cisplatin (100 mg/m<sup>2</sup>) and epirubicin (100 mg/m<sup>2</sup>) combination were given before RT according to the study protocol.

### Scoring of Mucosal Toxicity

Objective and subjective evaluations of the mucosal reactions to RT were performed (Table 2). During objective assessments, weekly physical examinations were done and the changes in oral mucosa were graded according to the Radiation Therapy Oncology Group (RTOG) mucosal reaction scoring.

### Oral GM-CSF Treatment

Oral GM-CSF treatment was initiated in patients who developed G III-IV mucositis with a

regimen of 400 µg GM-CSF dissolved in 250 ml water and consumed in 24 hrs as 4 times in a day. At each rinsing, this mouthwash solution was held in the mouth for 3-5 minutes. After five days of therapy, mucositis was reassessed. If the patient's mucositis was not reduced to G II mucositis or the patient wished to continue the treatment, a second five-day regimen was started.

### Statistical Method

Chi-square test was used to assess the relation between the different parameters and the severity of mucositis. Correlation test was chosen to compare subjective and objective evaluation results.

## RESULTS

Grade III-IV mucosal toxicity was observed in 22 patients (68%) (Table 1). Thirteen of the G III-IV mucositis cases were male. No statistically significant correlation was present between gender differences and the severity of mucositis ( $p=0.92$ ). Furthermore, mucositis pathogenesis was not dependent on the stage of the cancer ( $p=1.0$ ). Similar findings were present between the anatomical location of the cancer and the development of mucositis (data not shown).

Twelve (67%) of the postoperative and 10 (72%) of the only-biopsy patients developed severe mucositis, and these percentages were not significantly correlated to the severity of mucositis ( $p=0.77$ ) (Table 1). G III-IV mucosal toxicity was seen in 11 out of 12 smokers (92%) and 11 out of 20 non-smokers (55%), and there was significant difference between the groups with respect to development of severe mucositis during RT ( $p=0.04$ ) (Table 1).

**Table 3**

Radiation dose in Grade III-IV mucositis cases and response to GM-CSF treatment

Dose (Gy)	Mucositis grade	5th day	10th day
66	4	2	2
45	4	3	2
54	4	2	2
48	4	2	1
26	3	2	1
46	4	3	2
46	4	2	2
60	4	3	2
60	4	3	2
50	4	2	2
44	3	2	2
34	3	2	1
46	4	3	2
50	3	2	1
20	3	2	-
50	4	2	-
60	3	2	-
66	4	2	-
60	3	2	-
46	3	2	-
20	3	3	-
42	3	2	-

**Table 4**

Correlation of objective and subjective responses

Subjective response	Objective response		
	PR	CR	NR
PR	12	3	-
CR	1	4	-
NR	1	-	1

PR: Partial response; CR: Complete response; NR: Non-response.

When the ratio of involved RT field and the dosage of the given radiation were compared with the severity of mucositis, there was no statistically significant correlation among these variables.

Microbial investigation was performed in 19 of the 22 patients who developed GIII-IV mucositis. Oral microflora was normal in 10 patients (52%). The remaining 9 (48%) patients presented gram-negative cocci. Among those, 3 patients also had *Pseudomonas aeruginosa*, 2 patients *Acinetobacter*

*spp*, 2 patients *Klebsiella pneumoniae*, 1 patient *K. pneumoniae* and *Enterobacter spp*, and 1 patient *P. aeruginosa* and *Acinetobacter spp* in their oral flora. In 7 out of 10 patients (70%) who developed G I-II mucositis and in 3 out of 10 patients (30%) who had no GM-CSF treatment, oral flora was normal and was dominated with gram-negative cocci.

Grade IV mucositis was seen in 12 patients and G III in 10 patients (Table 3). Among these patients, five-day GM-CSF regimen decreased the toxicity by one grade in 14 (64%) patients and by two grades in 7 (32%) patients, when objective assessment criteria were used (Table 3). One patient did not respond to the treatment. During 10-day GM-CSF treatment, 10 patients with G IV mucositis who showed improvement by one or two grades following five-day GM-CSF regimen, continued to show further improvement.

In the subjective assessment, pain was abolished in 5 (23%) patients completely and in 15 (68%) patients partially. Two (9%) patients showed no subjective response to GM-CSF. In objective response, one-grade improvement was interpreted as partial response and two-grade improvement was interpreted as complete response. Objective and subjective responses were compared (Table 4). Four (80%) patients who reported complete improvement in pain and dysphasia also had complete objective improvement. Twelve (80%) patients who reported partial subjective improvement also had partial objective improvement. The objective and subjective responses were well correlated ( $\kappa = 0.54$ ), and in 17 (77.2%) patients, the same degree of objective and subjective improvements were obtained.

There was no interruption in RT because of mucosal toxicity and the RT was completed as planned. Weekly weight measurements showed that 4 (18%), 5 (23%) and 13 (59%) of the patients lost >10%, 5-10% and <5% of his/her baseline weight, respectively.

Weekly neutrophil counts increased in 7 patients and decreased in 1 patient. In the majority (64%) of the patients, neutrophil counts were unchanged even during mucositis. After GM-CSF treatment,

neutrophil count increased in only 6 patients and there were no changes in hematologic variables in 16 (73%) patients.

## DISCUSSION

Radiation induces mitotic death in oral mucosal basal cells, thus causing acute mucosal reactions.<sup>[13,14]</sup> In addition, RT disrupts the integrity of desmosomes between mucosal cells and increases the traumatic effects of even normal functions such as feeding.<sup>[13]</sup> The time to the development of mucosal reactions and the grade of such reactions are influenced by individual systemic factors such as intrinsic radiosensitivity, diabetes, collagen diseases, cigarette smoking and alcohol consumption, genetic make-up, and socioeconomic conditions besides therapeutic factors like the radiation dose used and radiated tissue volume.<sup>[15]</sup> The negative effect of additional neoadjuvant CT or concomitant CT has been reported as well. In our study, only cigarette smoking had a statistically significant effect on mucositis ( $p=0.04$ ). Although the size of the radiated area had no significant effect on the grade of mucositis, a trend for a higher chance to develop G III-IV mucositis with larger surface area was present (e.g. 58% vs 83%).

It has been reported that the conventional fractionation scheme has increased probabilities of erythema and pseudomembrane formation.<sup>[13,14]</sup> Similar to the other studies, we determined the presence of G III-IV mucositis developing at a median 50 Gy (range: 20-66).

Radiotherapy decreases the amount and changes the quality of saliva. Thus, the protective effects of saliva decrease and changes in oral microflora could occur.<sup>[15]</sup> Abnormal gram-negative colonization in the oral cavity is one of the predisposing factors for radiation-induced mucositis.<sup>[16]</sup> In our baseline oral cultures, there was only one case with *Candida* colonization but the culture results of G III-IV mucositis cases showed that 45% of them had gram-negative coccus proliferation. This percentage was only 30% in G I-II mucositis, suggesting that the gram-negative colonization in mucositis may play a role in the progression of mucosal reaction.

Various agents such as systemic analgesics, local anesthetics, mouthwash solutions including acetylsalicylic acid, and steroids have been used in the symptomatic treatment of acute mucositis.<sup>[17-19]</sup> Sucralfate- and benzydamine-containing preparations have also been as effective.<sup>[20-22]</sup> These agents may decrease the pain temporarily with their anesthetic effects and may improve nutritional uptake.

Granulocyte macrophage colony-stimulating factor (GM-CSF) is a cytokine effective on growth and proliferation of hemopoietic cells like neutrophils and macrophages, and it has similar effects on non-hemopoietic cells, namely fibroblasts in bone marrow and endothelial cells.<sup>[23]</sup> It has also been shown to be effective on proliferation of keratinocytes both *in vitro* and *in vivo*.<sup>[24]</sup> The perilesional, intradermal, subcutaneous, or topical application of GM-CSF could accelerate wound healing in hereditary hemoglobinopathies, chronic ulcers of Behçet's disease, decubitus ulcers, venous or arterial lower extremity ulcers, non-healing postoperative wounds, burns, skin grafts, and Kaposi sarcoma.<sup>[25-29]</sup>

*In vitro* studies report that at the cellular level, the expression of GM-CSF receptors in gingival fibroblasts can be upregulated in the presence of GM-CSF in a dose-dependent manner, and some cellular activities such as modelling of cell skeleton and fibronectin production may be modulated.<sup>[30,31]</sup> Further, the mammalian cells respond to the RT-induced oxidative stress by the activation of the genes coding GM-CSF and interleukin (IL)-1.<sup>[32,33]</sup>

Several assumptions on the possible mechanisms of systemic GM-CSF use for mucositis treatment exist at present. GM-CSF decreases the duration of mucositis in patients with whole body radiation plus stem cell transplantation.<sup>[34]</sup> It has also been reported that GM-CSF demonstrates no tumor-stimulating effect when used systemically or subcutaneously.<sup>[35,36]</sup> Kannan et al.<sup>[9]</sup> reported that in patients with head and neck carcinoma, side effects like mucositis, pain and functional disorders were rare or minimal following simultaneous use of subcutaneous GM-CSF during RT. Similarly, in a study of Wagner et al.,<sup>[11]</sup> patients diagnosed as locally advanced head and neck carcinoma and

treated with adjuvant RT together with subcutaneous GM-CSF had a significant decrease in pain and in the grade of mucositis.

Studies on topical use of GM-CSF as a mouthwash during RT are limited. Rovirosa et al.<sup>[12]</sup> reported that GM-CSF mouthwash solution was effective in mucosal ulcerations due to RT and improved the pain, nutritional uptake and weight loss. We evaluated the efficacy of GM-CSF mouthwash solution in G III-IV mucositis cases, according to objective and subjective criteria. In the subjective assessment, 23% complete and 68% partial response and in the objective assessment, 32% complete and 63% partial response were obtained. From a statistical point of view, the same results were obtained by using objective and subjective evaluation criteria in 77.2% of the patients, and there was a moderate parallelism between the subjective and objective response rates.

Subjective evaluation helps to assess the quality of life of the patients and it is also important in patient compliance. A decrease in or disappearance of symptoms like pain and dysphagia prevents the interruptions. In our study, there was no need to interrupt the RT for patients using GM-CSF mouthwash solution. The completion of the treatment for the pre-planned duration improves local control rates significantly. Thus, the symptomatic treatment of mucositis during RT also increases the success of the therapy in addition to facilitating a decrease in the acute toxicity.

There is a need to investigate the effectiveness of local GM-CSF treatment compared to the other topical agents used in the literature. The stability of neutrophil counts and other blood parameters shows that systemic effects due to mucosal absorption are absent or minimal. The mechanism(s) of these protective effects may be related to the modulation of local immune response, such as an increased turnover rate of oral epithelial cells, activation of functions and collagen deposition and/or neovascularization.

Understanding the roles of humoral and cellular factors in the pathogenesis of mucosal reactions, and the molecular interactions between those fac-

tors and GM-CSF, will improve the treatment of mucositis associated with RT and other cytotoxic therapies.

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