Cancer Chemother Pharmacol (2001) 47 (Suppl): S23–S28

ORIGINAL ARTICLE

**Malook S. Gujral · Pravas M. Patnaik
Rashmi Kaul · Hemen K. Parikh · Christian Conradt
Chetan P. Tamhankar · Gautam V. Daftary**

Efficacy of hydrolytic enzymes in preventing radiation therapy-induced side effects in patients with head and neck cancers

Abstract *Purpose*: Based on in vitro and on clinical evidence of protection against acute side effects of radiation, a prospective randomized, open study was performed to determine the efficacy of an oral proteolytic enzyme preparation in patients with head and neck cancer receiving conventional fractionated radiation therapy. *Methods*: Patients with stage T3/T4 head and neck cancer were eligible. One hundred patients from two centres were entered into the study. ^{60}Co gamma-radiation was delivered at a standard daily radiation dose of 2 Gy in 25–35 fractions over a period of 6–7 weeks. Two lateral parallel opposing fields were used with a portal area of 10 × 15 cm. Patients assigned to the test group arm additionally received enzyme tablets orally t.i.d. starting 3 days prior to radiation therapy, and continuing up to 5 days after completion of the course of radiation therapy. Patients in the control arm were not given any drug or placebo. Acute radiation side effects were described as mucositis, skin reaction, dysphagia, and were graded at each visit during and after radiation therapy, following RTOG/EORTC criteria. *Results*: The severity (maximum extent) of acute radiation therapy side effects was significantly less in enzyme-treated patients than in control patients: mucositis (mean: 1.3 vs 2.2, $P < 0.001$), skin reaction (1.2 vs 2.4, $P < 0.001$) and dysphagia (1.4 vs 2.2, $P < 0.001$). The duration of these side effects as well as the sum scores of side effects were also less in the study arm. *Conclusions*: Combination of enzyme therapy with conventional fractionated radiation therapy was feasible and well-tolerated. There was significant protection against acute side effects of radiation therapy in the study arm. Not only was the severity of acute side effects less but the duration was shorter and the time to onset was also delayed. Prospective randomized double-blind studies would verify this role of an oral enzyme therapy as standard co-medication with radiation therapy to the head and neck region.

Key words Radiation therapy · Mucositis · Enzyme therapy · Acute reaction · Side effects · Supportive care

Work presented at ECCO 10, The European Cancer Conference, Vienna, 12–16 September 1999

M. S. Gujral · R. Kaul
SGPT Cancer Hospital, M.Y. Hospital Campus,
Indore, India

P. M. Patnaik
AH Regional Cancer Centre, Cuttack, India

H. K. Parikh
Tata Memorial Hospital, Parel, Mumbai, India

C. Conradt
Department of Medical Biometry,
University of Heidelberg, Germany

C. P. Tamhankar · G. V. Daftary
SIRO Research Foundation, Thane, India

G. V. Daftary (✉)
SIRO Research Foundation, 31, Maker Chambers VI,
Nariman Point, Mumbai 400 021, India
Tel.: +91-22-2026295; Fax: +91-22-2886883
e-mail: siro@vsnl.com

Introduction

In patients with squamous cell carcinoma of the head and neck region, acute radiation therapy side effects are common. Oral mucositis results from impairment of epithelial cell production as a consequence of radiation-induced cell kill.

The threshold for confluent mucositis of human oral mucosa following fractionated radiation is about 20 Gy. Confluent acute mucositis occurs in up to 95% of patients after a radiation dose of 30 Gy. This often leads to compromised dose intensity or interruption of therapy, and hence reduces the response rate, and affects overall survival. Moreover, marked acute effects may well result in consequential late sequelae [1–5].

Therapeutic interventions that prevent or attenuate acute radiation therapy side effects therefore are of

potential benefit [6]. Although many available approaches have been tested to treat this morbidity, none has been found to be a definite benefit [7–11]. In parallel to the epithelial response a vascular, inflammatory component is observed in oral mucositis too. This is associated with deregulated levels of cytokines with various consequences. Several studies have evaluated the efficacy of proteolytic enzymes in the management of post-operative edema and inflammation [12–16]. The analgesic and anti-inflammatory effects of hydrolytic enzymes such as trypsin and chymotrypsin have been observed in post-operative traumatic inflammation and in experimental radiation therapy [17, 18]. The present work reports a prospective, randomized, open trial to study the supportive effect of a proteolytic enzyme-combination in prevention of acute side effects of radiation therapy in patients with head and neck cancer.

Patients and methods

This study was carried out between August 1996 and April 1997 at two UICC approved centres: the SGPT Cancer Hospital at Indore, Madhya Pradesh, and the AH Regional Cancer Centre at Cuttack, Orissa. The study was carried out after approval had been obtained from the responsible Institutional Ethics Committees of each centre.

Patients

A total of 100 adult patients (aged 18–65 years) was recruited for the study. Inclusion criteria were biopsy-proven squamous cell carcinoma of the head and neck – oral cavity or oropharynx staged T1-T3 at subsites, alveolo-buccal, oral tongue, base of tongue, epiglottis/vallecula, tonsil and pharyngeal wall (lateral or posterior). Previous chemotherapy was allowed, but no prior radiation therapy. Patients with distant metastasis, Karnovsky Index < 70 or altered hematological or biochemical parameters were not eligible for participation in the study. Patients written informed consent was obtained before their enrolment in the study.

Study plan

Radiation therapy. Patients received 50 to 70 Gy telecobalt therapy (Theratron) using a standard daily radiation dose of 2 Gy in 25–35 fractions over a period of 6–7 weeks. Two lateral parallel opposing fields were used with a portal area of 10×15 cm.

Randomization procedure. Patient randomization was carried out by the "sealed envelope" method. At each centre, 50 patients were to be randomized, assigning 25 patients to each arm. The patients were enrolled in chronological order. They were assigned consecutive patient numbers, and received either radiation therapy, or radiation therapy together with enzyme therapy.

Study drug administration. Before the start of radiation therapy all the patients were advised to maintain high oral hygiene after a complete dental check-up. No prophylactic treatment aimed at mucositis was used. Non-steroidal anti-inflammatory drugs such as diclofenac and ibuprofen were prescribed only as "rescue medication" when patients reported moderate pain and patchy mucositis. In cases of severe pain and confluent mucositis, morphine analogues such as dextropropoxyphen and steroids were used. Patients belonging to the study arm were given the enzyme-combination product (Wobe-Mugos E, MUCOS Pharma, Geretsried, Germany), containing papain (100 mg), trypsin (40 mg) and chymotrypsin (40 mg). Three tablets were given orally three times a day from 3 days prior to start of radiation therapy until 5 days after completion of radiation therapy. Patients in the control arm were not administered the drug or placebo. Patients' compliance to treatment was ensured by "pill counting".

Evaluation

Evaluation of tissue responses for acute radiation side effects was done by grading mucositis, dysphagia, and skin reactions at each visit. Grading was by RTOG/EORTC criteria: mucositis grade 0 = nil, grade 1 = mild, grade 2 = moderate, grade 3 = severe; dysphagia grade 0 = nil, grade 1 = for solids, grade 2 = for semi-solids, grade 3 = for liquids, grade 4 = requiring ryles tube/feeding gastrostomy; skin reactions grade 0 = nil, grade 1 = erythema, grade 2 = early desquamation/pigmentation, grade 3 = moderate dry/early moist desquamation, grade 4 = blister formation/skin peel. At each centre, patients were initially evaluated for baseline mucositis, dysphagia and dermatitis. Evaluation was continued at weekly intervals for 6 to 8 weeks covering the period of radiation therapy, and for another 5 to 6 months after the end of radiation therapy. The maximum scores for the respective observation time were recorded. Grading was always done by the same investigator.

Statistical methods

The primary efficacy variables were defined as the maximum severity of mucositis, dysphagia and skin reactions during a planned period of radiation therapy. In addition, time until the maximum toxicity scores were reached, sum-scores during the first 4 weeks of radiation therapy, and overall sum-scores were analysed. The Wilcoxon rank-sum test (two-sided) was used to test for treatment differences. In order to account for multiple tests of significance ($n = 3$), the crude P values were adjusted according to the step-down method of Bonferroni-Holm. The secondary variables were evaluated descriptively. To assess the homogeneity of the treatment groups with respect to certain characteristics, the Wilcoxon rank-sum test was used to test for differences in distribution of continuous variables, whereas for categorical variables Fisher's exact test was used. Statistical analysis was performed with SAS programmes (SAS Institute, Cary, N.C., USA).

Results

Patients

A total of 100 patients was enrolled, and randomized at both centres. This included 53 in the study arm (25 at Indore, and 28 at Cuttack) and 47 in the control arm (25 at Indore, and 22 at Cuttack). The site of primary disease was alveolo-buccal in 44 patients (23 in control, 21 in study arm), tongue/base of tongue in 31 patients (12 in control, 19 in study arm) and 25 (12 in control, 13 in study) at other sites such as tonsils and epiglottis. There was no statistically significant difference observed between the control and the test groups with respect to the demographic characteristics of the patients (Table 1). Both groups received comparable cumulative doses of radiation therapy. Control-arm patients received 58.6 ± 8.8 Gy over 45 ± 9 days, while study-arm patients received an average dose of 59.1 ± 6.2 Gy over 45 ± 9 days. Patients in the test group additionally received enzyme therapy over a period of 54 ± 9 days, with treatment starting 4 ± 2 days before radiation therapy.

Two patients, one from each arm, did not complete the study for personal (non-medical) reasons. Radiation therapy was generally on an outpatient basis, however, at Cuttack centre with a wider catchment area, 33 patients (19 enzyme group, 14 control group) were hospitalized in order to ensure their compliance with radiation therapy. There were radiation therapy gaps in a total of 14 patients. Twelve of these gaps were for social/technical reasons (three from the control arm and nine from the study arm). In two patients, radiation had to be temporarily discontinued due to severe radiation therapy-related reactions. Both these patients belonged to the control arm.

Efficacy evaluation

The maximum extent of acute radiation side effects such as mucositis, skin reactions and dysphagia was lower in enzyme-treated patients than in control patients (Table 2, Fig. 1). Mucosal reactions were graded about one grade lower, and the number of patients with severe mucositis and problems in swallowing was considerably less. In none of the patients of the enzyme group were skin reactions of grade IV (blister formation/skin peel) observed (Table 3). These findings were consistent at both centres, and were found to be statistically significantly different.

Secondary criteria for efficacy were the areas under the curves in graphs for mucositis, skin reactions, and dysphagia (Table 4), and the time to reach a certain grade after commencement of radiation therapy. Table 5 displays the results for toxicity grades with comparable patient numbers, showing that the same grade of mucositis, skin reactions, and dysphagia was reached later in patients receiving additional enzyme therapy.

Disease response at the end of radiation therapy (end of week 8) is shown in Table 6. In both groups a comparable rate of complete and partial remissions was observed, but the proportion of patients with complete regression was slightly higher in the enzyme group. At last follow up (visit 11, 5 to 6 months after end of radiation therapy) two-thirds of the patients could be evaluated. There were 42 patients with complete remission, 18 patients had partial remission, and seven patients had progressive disease. The distribution between the two groups was similar. Two patients died (both belonging to the study arm), one due to cardiac arrest in the sixth week of the study, and the other due to bleeding from the site of the primary disease after completion of radiation therapy.

General safety

The reported adverse events not directly related to radiation therapy included pain/body ache, fever, weakness, vomiting, itching, hemoptysis and swelling. These were generally mild and of short duration. Their overall incidence was not significantly different between the two arms, and thus it was not possible to relate any of the reported side effects directly to the test drug.

Table 1 Patient characteristics at baseline

	Enzyme group $n = 53$	Control group $n = 47$	P value
Age (years)	50.3 ± 9.4	51.2 ± 11.2	0.32
Gender (male)	34	35	0.29
Body weight (kg)	59.7 ± 10.2	57.9 ± 9.8	0.54
Height (cm)	150.9 ± 16.4	148.6 ± 13.3	
Site of disease			
Alveolo-buccal	21	23	
Tongue	19	12	
Others	13	12	
T stage			
0	2	4	0.39
1	1	3	
2	26	18	
3	13	8	
4	11	14	
N stage			
0	17	18	0.63
1	23	18	
2	10	11	
3	2	0	
x	1	0	

Discussion

Oral mucositis continues to be a major morbidity in the absence of well-established agents to prevent the acute

Table 2 Maximum extent of acute side effects during radiation therapy

	Therapy	n	Minimum	Maximum	Mean	Standard deviation	Mean difference	95% CI	P value[a]
Mucositis	Radiation therapy	46	1	3	2.24	0.60	0.92	0.67–1.17	< 0.0001
	Radiation therapy + enzymes	53	0	3	1.32	0.64			
Skin reactions	Radiation therapy	46	1	4	2.39	1.11	1.16	0.80–1.53	< 0.0001
	Radiation therapy + enzymes	53	0	3	1.23	0.75			
Dysphagia	Radiation therapy	46	1	3	2.15	0.60	0.77	0.53–1.02	< 0.0001
	Radiation therapy + enzymes	53	1	3	1.38	0.63			

[a] Adjusted for multiple testing ($n = 3$) by step-down method of Bonferroni-Holm

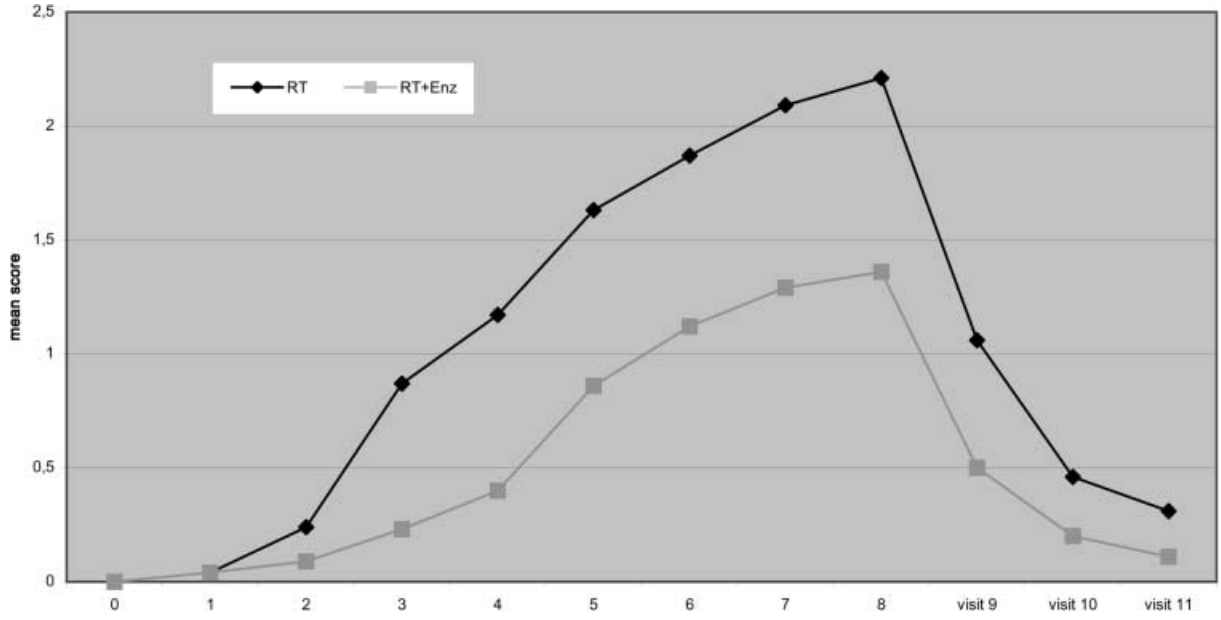

Fig. 1 Mean scores for mucositis (grade 0 = nil, grade 1 = mild, grade 2 = moderate, grade 3 = severe) for time during radiation therapy (weeks 1 to 8), and for time of recovery after end of radiation therapy (visit 9, 6–8 weeks; visit 10, 2–3 months; visit 11, 5–6 months, after end of radiation therapy)

effects of radiation, occurring in all patients by the fourth week of radiation therapy [19]. In the North Central Treatment Group and Mayo Clinic trial using chlorhexidine, very severe mucositis was seen in more than 50% of the cases [20]. Such side effects lead to unscheduled interruptions in radiation therapy, and consequently affect the final outcome of treatment since the patient receives reduced levels of the intended dose of radiation [19, 21].

Proteolytic enzymes have been demonstrated to have three potential mechanisms of action – modulation of the inflammatory cytokine cascade, reduction of TGF-β levels, and scavenging of free radicals [22, 23]. The basis of ionizing radiation is the formation of free radicals. Papain, one component of the enzyme-combination product investigated here, has been shown to have clinically significant activity as a free-radical scavenger. High serum TGF-β_1 levels were shown to be correlated with the development of fibrosis in patients with breast cancer who have received radiation therapy [24]. Tissue injury after radiation is associated with deregulation of cytokines (TNF-α, IL-2 and IL-1β) and over-expression of various molecules like ICAM-1, selectins and

Table 3 Maximum extent of radiation therapy-induced acute toxicity, frequency distribution

	Therapy	Grade 0	Grade I	Grade II	Grade III	Grade IV
Mucositis	Radiation therapy	0	4	27	15	–
	Radiation therapy + enzymes	2	35	13	3	–
Skin reactions	Radiation therapy	0	11	17	7	11
	Radiation therapy + enzymes	8	27	16	2	0
Dysphagia	Radiation therapy	0	5	29	12	0
	Radiation therapy + enzymes	0	37	12	4	0

Table 4 Area under the curve for acute mucosal reactions for weeks 1 to 8 during radiation therapy, including only patients with complete data

	Therapy	*n*	Minimum	Maximum	Mean	Standard deviation	*P* value
Mucositis	Radiation therapy	43	2	18	10.2	3.6	< 0.0001
	Radiation therapy + enzymes	50	0	19	5.4	3.6	
Skin reactions	Radiation therapy	43	3	17	9.5	3.9	< 0.0001
	Radiation therapy + enzymes	50	0	14	3.9	2.9	
Dysphagia	Radiation therapy	43	5	21	10.1	3.6	< 0.0001
	Radiation therapy + enzymes	50	1	18	5.2	3.4	

Table 5 Time to respective side effect grade in weeks for acute mucosal reactions for weeks 1 to 8 during radiation therapy, given for the highest grade with comparable patient numbers

	Therapy	*n*	Mean	Standard deviation	*P* value
Mucositis Grade II	Radiation therapy	27	5.7	1.2	0.0014
	Radiation therapy + enzymes	13	6.9	0.8	
Skin reactions Grade II	Radiation therapy	17	5.7	1.4	0.048
	Radiation therapy + enzymes	16	6.6	1.6	
Dysphagia Grade I/II	Radiation therapy	5/29	3.6/6.1	0.5/1.3	0.0092/0.0064
	Radiation therapy + enzymes	37/12	5.2/7.3	1.5/0.8	

Table 6 Disease response evaluated as complete/good, moderate (partial), and poor/nil response for disease progression at end of radiation therapy and at end of follow-up, e.g. 5 to 6 months after end of radiation therapy

	Therapy	Patients lost for evaluation	Complete/ good response	Moderate response	Poor/no response/ progression	*P* value[a]
Visit 8:8 weeks after start of radiation therapy	Radiation therapy	4	23/15	5	0	0.23
	Radiation therapy + enzymes	3	32/16	1	1	
Visit 11:5–6 months after end of radiation therapy	Radiation therapy	18	17/9	1	2	0.76
	Radiation therapy + enzymes	15	25/7	1	5	

[a] *P* value based on Wilcoxon rank-sum test for an ordinal response-scale

integrins. In consequence, increased migration of neutrophils to the site of injury and perpetuation of the inflammatory process are observed. Use of immunoregulatory systemic enzyme therapy has been demonstrated to down-regulate these over-expressed adhesion molecules, thereby reducing the inflammation [22].

The present study gives evidence of a role for proteolytic enzymes in preventing and reducing the acute side effects of radiation therapy in patients with squamous cell carcinoma of the head and neck region. Administration of a combination preparation of the proteolytic enzymes papain, trypsin, and chymotrypsin to patients at both medical centres resulted in a statistically significant reduction in the severity of mucositis, dysphagia and skin reaction. Significantly fewer patients progressed towards moderate and severe reactions in the study group than in the control group. Similarly, the onset of the side effects graded 2 and 3 was also delayed. Currently a larger randomized double-blind study is being conducted to verify this benefit.

Acknowledgements The authors thank Dr. W. Schiess (Mucos Pharma, Geretsried, Germany) for his contributions during study conduct and manuscript preparation.

References

1. Maciejewski B, Skladowski K, Pilecki B, Taylor JM, Withers R H, Miszczyk L, Zajusz A, Suwinski R (1996) Randomized clinical trial on accelerated 7 days per week fractionation in radiotherapy for head and neck cancer. Preliminary report on acute toxicity. Radiother Oncol 40: 137
2. Corvo R, Sanguineti G, Scala M, Garaventa G, Santelli A, Barbieri M, Vitale V (1994) Primary site as predictive factor of local control in advanced head and neck tumors treated by concomitant boost accelerated radiotherapy. Tumori 80: 135
3. Lusinchi A, Lartigau E, Luboinski B, Eschwege F (1994) Accelerated radiation therapy in the treatment of very advanced and inoperable head and neck cancers. Int J Radiat Oncol Biol Phys 29: 149
4. Epstein JB, Wong FL (1994) The efficacy of sucralfate suspension in the prevention of oral mucositis due to radiation therapy. Int J Radiat Oncol Biol Phys 28: 693
5. Wadleigh RG, Redman RS, Graham ML, Krasnow SH, Anderson A, Cohen MH (1992) Vitamin E in the treatment of chemotherapy-induced mucositis. Am J Med 92: 481
6. Posner MR, Weichselbaum RR, Fitzgerald TJ, Clark JR, Rose C, Fabian RL, Norris CMJ, Miller D, Tuttle SA, Ervin TJ (1985) Treatment complications after sequential combination chemotherapy and radiotherapy with or without surgery in previously untreated squamous cell carcinoma of the head and neck. Int J Radiat Oncol Biol Phys 11: 1887
7. Verdi CJ (1993) Cancer therapy and oral mucositis. An appraisal of drug prophylaxis. Drug Saf 9: 185
8. Marks JE (1997) Mucosal protectants and their application for head and neck chemoirradiation. Curr Opin Oncol 9: 267
9. Kannan V, Bapsy PP, Anantha N, Doval DC, Vaithianathan H, Banumathy G, Reddy KB, Kumaraswamy SV, Shenoy AM (1997) Efficacy and safety of granulocyte macrophage-colony stimulating factor (GM-CSF) on the frequency and severity of radiation mucositis in patients with head and neck carcinoma. Int J Radiat Oncol Biol Phys 37: 1005
10. Pfeiffer P, Madsen EL, Hansen O, May O (1990) Effect of prophylactic sucralfate suspension on stomatitis induced by cancer chemotherapy. A randomized, double-blind cross-over study. Acta Oncol 29: 171
11. Kuhrer I, Kuzmits R, Linkesch W, Ludwig H (1986) Topical PGE2 enhances healing of chemotherapy-associated mucosal lesions. Lancet 1(8481): 623
12. Gupta MK, Khanna JN, Khera SS, Fonseca JB (1980) Ars curandi. Odontology 7: 313–322
13. Love JW (1968) The effect of orally administered proteolytic enzymes on the postoperative course of periodontal surgery. J Periodontology 39: 337
14. Mourgues FD (1971) Preventive and corrective treatment of inflammatory edemas in dentistry and maxillofacial surgery, particularly by means of proteolytic enzymes of vegetable origin (in French). Chir Dent Fr 41: 31

15. Lie KK, Larsen RD, Posch JL (1967) Evaluation of an oral proteolytic enzyme in operations upon the hand. Surg Gynecol Obstet 125: 595
16. Garcia-Velasco J, Araico-Laguillo J (1972) Evaluation of the efficacy of proteolytic enzymes by oral route in the control of postsurgical inflammation of the hand (in Spanish). Arch Invest Med (Mex) 3: 7
17. McCue FCD, Webster TM, Gieck J (1972) Clinical effects of proteolytic enzymes after reconstructive hand surgery. A double-blind evaluation of oral trypsin-chymotrypsin. Int Surg 57: 479
18. Koberg W (1968) Prophylaxis and treatment of postoperative and posttraumatic edema in the jaw and face region (in German). Zentralbl Chir 93: 381
19. Van der Schueren E, Van den Bogaert W, Vanuytsel L, Van Limbergen E (1990) Radiotherapy by multiple fractions per day (MFD) in head and neck cancer: acute reactions of skin and mucosa. Int J Radiat Oncol Biol Phys 19: 301
20. Makkonen TA, Bostrom P, Vilja P, Joensuu H (1994) Sucralfate mouth washing in the prevention of radiation-induced mucositis: a placebo-controlled double-blind randomized study. Int J Radiat Oncol Biol Phys 30: 177
21. Adelstein DJ, Saxton JP, Van Kirk MA, Wood BG, Eliachar I, Tucker H, Mand Lavertu P (1994) Continuous course radiation therapy and concurrent combination chemotherapy for squamous cell head and neck cancer. Am J Clin Oncol 17: 369
22. Kokron E, Rehberger L, Desser L (1992) J Cancer Res Clin Oncol 118 [Suppl R117]
23. Lehmann PV (1996) Immunomodulation by proteolytic enzymes. Nephrol Dial Transplant 11: 952
24. Li C, Wilson PB, Levine E, Barber J, Stewart AL, Kumar S (1999) TGF-β1 levels in pre-treatment plasma identify breast cancer patients at risk of developing post-radiotherapy fibrosis. Int J Cancer 84: 155