Clin Oral Invest (2007) 11:293–296
DOI 10.1007/s00784-007-0119-y

ORIGINAL ARTICLE

Mucosal secretion changes during radiotherapy in the oral cavity

Luaay Aziz · Anders Ebenfelt

Received: 6 February 2007 / Accepted: 20 April 2007 / Published online: 24 May 2007

Abstract Mucositis in the oral cavity is a serious complication during radiation therapy for head and neck cancer, causing local discomfort and pain. In severe cases, hospitalization and interruption of radiotherapy may be necessary. The pathogenesis of this mucositis is not clear. With the purpose of getting more understanding of the pathogenesis of the mucositis, we examined the mucosal secretion from ten patients during radiotherapy with an imprint technique. In the secretion we studied the cellular composition and cellular function. In eight of ten treated patients the numbers of granulocytes increased in the secretion after 2 weeks of radiation therapy. The granulocytes, however, did not show any signs of phagocytosis. The patients all developed mucositis. We propose that the granulocytes in the secretion might play an important role in the development of mucositis during radiotherapy.

Keywords Mucosa · Mucosal secretions · Epithelial cells · Granulocytes radiotherapy

Introduction

Radiation therapy is an important method in the treatment of head and neck cancer. Radiation produces early and chronic effects on the oral mucosa. The early radiation effect causes local discomfort and pain and this can cause swallowing difficulties with nutritional problems [3, 9]. Severe mucosal effects will necessitate hospitalization and treatment of complications and may lead to interruption of radiotherapy [11].

Radiation effects are dose-related [1, 10]. Oral erythema is discernible 1 week [1] or 2 weeks [13] after the beginning of radiotherapy as the first sign of mucositis. After 2 weeks of therapy, approximately 20 Gy, the edema will be more pronounced and the erythema less obvious due to pressure on the capillaries resulting from the increased extravascular tissue. After 3 weeks and 30 Gy, there is an increase in the permeability of the small blood vessels and connective tissue leading to the connective tissue becoming edematous and the mucous membrane further stretched [2].

Radiation produces mucosal thinning, salivary gland atrophy [8], and vascular fibrosis. It is generally believed that cell death and inhibition of mitosis in the basal cell population, together with continuous cell loss from the mucosal surface, will cause mucosal thinning [12]. However, the pathogenesis is not really clarified and even if mitosis is inhibited in the mucosa and thus normal mucosa repair, there must be additional factors, which produce the inflammation.

Recent studies concerning other mucosal inflammations in the oral cavity have revealed the existence of an active cellular defense, consisting mainly of functionally active granulocytes in the secretion on the mucosal surface. These studies also strongly indicate that the granulocytes in the mucosal secretion are an important factor in the pathogenesis of mucosal inflammatory conditions [4].

This study was performed to investigate if there is an inflammatory and infectious process in the secretion on the oral mucosa in patients who undergo radiation therapy for oropharyngeal cancer. If so, this would increase our understanding of the pathogenesis of this condition.

L. Aziz · A. Ebenfelt (✉)
Department of Otorhinolaryngology, Head and Neck Surgery,
Sahlgrenska University Hospital,
413 45 Göteborg, Sweden
e-mail: anders.ebenfelt@orlss.gu.se

Table 1 Number of epithelial cells and granulocytes in five visual fields (×200) in the mucosal secretion in the controls

Subject	Epithelial cells	Granulocytes
A	223	0
B	154	30
C	116	0
D	140	2
E	66	0
F	70	0
G	119	0
H	600	0

Materials and methods

Ten consecutive patients with oral and oropharyngeal cancer (mean age 67 years), where the treatment chosen was radiotherapy, were included in the study. All patients received radiation at an average total dose of 61.2 Gy and the buccal mucosa was included in the radiation field in all patients. None of the patients was treated with steroids. As controls, eight patients (mean age 64 years) who were treated for noninflammatory conditions (epistaxis, vertigo, etc), and showed no clinical signs or symptoms for bacterial or fungal infection, were selected at our ENT department.

Samples for analysis were obtained from the patients before radiotherapy and 14 days after beginning the therapy. All patients then showed marked signs of mucositis with swollen, red, and painful mucosa (mucositis grade IV) [12]. In five patients we could obtain one further sample, which was taken at the end of therapy (6 weeks after first sample). The secretion was obtained from the buccal mucous membrane on the opposite site of the tumor and this location was in every case included in the radiation field. The sampling was done just before radiation was given. The samples were then analyzed by using an imprint technique [5]. Briefly, a piece of foam plastic, 23×15×15 mm, was firmly pressed against the buccal mucous membrane and then immediately pressed against a glass slide. The same side was used in every sampling occasion. The imprint was stained according to the May–Grünewald–Giemsa procedure after air drying and used for morphological and spatial studies of the cellular elements in the secretion. By light microscopy (Nikon, magnification ×40–400), the number of epithelial cells, clusters of coherent epithelial cells, and granulocytes were determined in five randomly selected visual fields in ×200. When big numbers of granulocytes were present in visual fields, it was impossible to count them exactly and thus, in these cases, the numbers were estimated. Presence of phagocytosis was determined in ten randomly selected visual fields in ×400.

From the controls, the samples were taken on the same location on the buccal mucosa. Only one sampling was done from the controls.

The results for the patients was compared with the controls by Mann–Whitney rank test.

The difference in results between the first and second samples in the patients was compared with Wilcoxon signed rank test.

The study was approved by the Committee of Research Ethics, Göteborg University.

Results

In the samples from the control group, we observed large numbers of epithelial cells, single cells, and clusters. Granulocytes were observed in two of eight controls but in small numbers. Phagocytosis was never observed.

In the samples from the tumor patients taken before radiotherapy, epithelial cells were observed in about the same numbers as in the controls. Granulocytes were observed in four of the ten patients but in small numbers compared to the numbers of epithelial cells. Phagocytosis was never observed.

Table 2 Number of granulocytes and epithelial cells in five visual fields (×200) in the mucosal secretion before and after 2 weeks radiotherapy

Patients	Before radiotherapy		After 2 weeks radiotherapy	
	Granulocytes	Epithelial cells	Granulocytes	Epithelial cells
A	0	246	1,000	15
B	217	205	67	46
C	17	78	0	10
D	57	58	1,000	43
E	0	341	1,000	32
F	2	44	1,000	0
G	0	27	1,000	5
H	0	48	25	28
I	0	25	71	8
J	0	30	90	0

Table 3 Number of granulocytes and epithelial cells in five visual fields (×200) in the mucosal secretion before and 6–8 weeks after radiotherapy

Patient	Before radiotherapy		After 6–8 weeks radiotherapy	
	Granulocytes	Epithelial cells	Granulocytes	Epithelial cells
D	57	58	2,000	3
E	0	341	0	46
F	2	44	0	18
G	0	27	113	3
H	0	48	250	4

There were no significant differences between patients and controls in the first sample concerning numbers of epithelial cells or granulocytes (Mann–Whitney rank test).

After 2 weeks of radiotherapy, there was a significant decrease in the number of epithelial cells in the samples ($p<0.01$, Wilcoxon signed rank test). In five of the patients there was a great increase in granulocytes, in three of the patients a moderate increase in granulocytes while in the other two there was no change in number of granulocytes. The change in numbers of granulocytes between first and second samples was significant ($p<0.03$, Wilcoxon signed rank test). Phagocytosis was not present in the second samples.

In three of the five patients where a third sample was taken after 6–8 weeks of treatment, the samples showed a marked decrease in the numbers of granulocytes compared with the samples taken after 2 weeks of treatment. These three patients all had high numbers of granulocytes in the sample taken after 2 weeks of treatment, while the remaining two patients still had high numbers of granulocytes in their third sample.

The numbers of epithelial cells and granulocytes in subjects and controls are summarized in Tables 1, 2, and 3.

Discussion

Mucositis is considered partly to be caused by mucosal thinning, which in turn is considered to be caused by cell death and inhibition of mitosis and by extravasation of fluid, causing edema [12]. Our study verified some of these changes by observation of decreasing numbers of epithelial cells and at the same time increasing numbers of granulocytes. Cell death and mitosis inhibition might thus explain the decreasing numbers of epithelial cells. Still, cell death and inhibition of mitosis does not explain inflammation.

In five of ten treated patients, huge numbers of granulocytes were present in the secretion after 2 weeks radiation therapy, and further three patients showed a marked increase in the number of granulocytes. The numbers of granulocytes in the secretion in these patients are in the same range as earlier described concerning patients with acute mucosal infection [4]. Thus, it seems plausible that the granulocytes in the secretion are at least partly responsible for the inflammation seen in the patients, with their red swollen mucosal surfaces. There are however other recent studies where it is found that irradiation causes infiltration in the oral epithelium mainly by mononuclear leukocytes [6, 7], whereas the number of granulocytes in the mucosa in those studies did not show any significant alteration compared with the number before irradiation [6, 7]. The difference between our findings in the mucosal secretion and the findings in the mucosal tissue in these studies could be because the granulocytes migrate immediately to the secretion instead of gathering in the epithelium.

One explanation for the presence of granulocytes in the secretion could be that irradiation causes damage and rupture of vessels in the mucosa, with passive leakage of blood, but this does not seem probable, as we did not observe any increase in numbers of other leukocytes or erythrocytes. Instead, it seems probable that the granulocytes in the secretion are present as a result of some chemotactic factor.

It is notable that we did not find any sign of phagocytosis in the samples with huge numbers of granulocytes. In the above referred study concerning other mucosal inflammations [4], large numbers of granulocytes invariably phagocytized bacteria, and one would have expected this to be the case in this study, too. The absence of phagocytosis in patients with mucositis means that we found no evidence of infection in the mucosal secretion. The mucositis after radiation therapy can thus be caused by other mechanisms than infection.

As granulocytes and their products can be toxic, not only to microorganism but also to the mucosal epithelium, it might be that the granulocytes play an important role in the development of mucositis seen during radiation therapy. More knowledge about the role of granulocytes in the mucosal secretion therefore seems to be urgently required for a better understanding of the pathogenesis of radiation mucositis.

In conclusion, we have found that during radiotherapy, big numbers of granulocytes are present in the mucosal secretion when the patients develop mucositis. Phagocytosis of bacteria is however not seen.

References

1. Al-Tikriti U, Martin MV, Branley PA (1984) A pilot study of the clinical effects of irradiation on the oral tissues. Br J Oral Maxillofac Surg 14:77–86
2. Baker DG (1982) The radiobiological basis for tissue reactions in the oral cavity following therapeutic x-irradiation. A review. Arch Otolaryngol 108:21–24

3. Beumer J, Silverman S, Benk SB (1972) Hard and soft tissue necrosis following radiation therapy for oral cancer. J Prosthet Dent 27:640–644
4. Ebenfelt A, Lundberg C (1996) Cellular defense in surface secretion in acute pharyngotonsillitis. Acta Otolaryngol 116:97–103
5. Ebenfelt A, Geterud Å, Granström G, Lundberg C (1995) A novel method for studies of cell-kinetics and spatial relations between leukocytes, epithelial cells and bacteria in the secretion on the surface of the mucosa. Acta Otolaryngol 115:106–111
6. Handschel J, Prott F-J, Sunderkötter C, Metze D et al (1999) Irradiation induce increase of adhesion molecules and accumulation of β2-integrin-expressing cells in humans. Int J Radiat Oncol Biol Phys 45(2):475–481
7. Handschel J, Sunderkötter C, Prott F-J, Mayer U et al (2001) Increase of RM3/1-positive macrophages in radiation induced oral mucositis. J Pathol 193:242–247
8. Hannig M, Dounis E, Henning T, Apitz N, Stosser L (2006) Does irradiation affect the protein composition of saliva? Clin Oral Invest 10:61–65
9. Kam AY, McMillan AS, Pow EH, Leung KC, Luk HW (2005) A preliminary report on patient acceptance of a novel intra-oral lubricating device for the management of radiotherapy-related xerostomia. Clin Oral Invest 9:148–153
10. Kashima HK, Kirkham WR, Andrews JR (1965) Post-irradiation sialoadenitis. Am J Roentgenol Radium Ther Nucl Med 94:271–291
11. Lockhart PB (1986) Oral complications of radiation therapy. In: Peterson D, Elias J, Sonis S (eds) Head and neck management of the cancer patient. Martinus Nijhoff, Boston, pp 429–449
12. Parulekar W, Mackenzie R, Jordan CK (1998) Scoring oral mucositis. Oral Oncol 34:63–71
13. Robinson JE (1976) Characteristics of irradiated soft and hard tissue. Prosthet Dent 35:549–552