ENHANCEMENT OF MYELOPEROXIDASE ACTIVITY IN WBCS IN ORAL CANCER PATIENTS TREATED WITH GRANULOCYTE MACROPHAGE COLONY STIMULATING FACTOR (GM-CSF) - A PILOT STUDY

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ABSTRACT

In a pilot study with five oral cancer patients undergoing radiotherapy (RT) three were given Granulocyte Macrophage Colony Stimulating Factor (GM-CSF) as a protective agent to reduce the mucosal inflammation during radiotherapy. The myeloperoxidase (MPO) enzyme activity in WBC was quantitated. The three patients showed a significant increase in the MPO activity when compared with two untreated controls indicating the efficacy of GM-CSF as a protective agent. It is suggested that further detailed studies with larger number of patients would be useful.

KEY WORDS: Myeloperoxidase, GM-CSF, Radiotherapy, Superoxide radicals, Neutrophils.

INTRODUCTION

As radiotherapy depresses the immune system, bacterial invasion and inflammation are very common side effects during radiotherapy of oral cancer. Granulocyte Macrophage Colony Stimulating Factor (GM-CSF) is a potent stimulator of neutrophils which showed beneficial effects in head and neck cancer(1). Hence, we have taken up a pilot study to assess the effect of GM-CSF in three patients undergoing radiotherapy. GM-CSF leads to an increased production of free radicals (superoxide radicals), which are detoxified *in vivo* by the enzymes myeloperoxidase and superoxide dismutase. The effect of GM-CSF on the oxidative metabolism of neutrophils has been previously studied (2,3). The action of GM-CSF is presumed to be through a receptor, details of which are under investigation. Although it has been conclusively proved that GM-CSF is active both *in vivo* and *in vitro* conditions, the mechanism of action has not yet been elucidated.

MATERIALS AND METHODS

Because GM - CSF is very expensive, only five patients with oral cancer were selected for the study, details of which are shown in Table 1. The test group of three patients were administered GM-CSF. Two patients who were not administered the GM-CSF served as controls. Two normal controls were also selected for study. All patients underwent the standard radiotherapy (6000 rads at 30 divided doses in 5 weeks). Within two weeks of receiving radiotherapy all of them developed very severe inflammation in the oral cavity. After 2 weeks of radiation, that is in the middle of the course of radiotherapy, the patients in the test group were given 1 µg/Kg body weight of GM-CSF, (supplied by Sandoz), subcutaneously daily for a period of six days. The administration was well tolerated by all patients. The GM-CSF administration resulted in marked reduction of local infection and remarkable toleration to radiotherapy. Blood samples were withdrawn from the patients in heparinised syringes at three stages of therapy: (1) prior to starting radiation, (2) after two weeks of radiation, before starting GM-CSF and (3) on completion of radiation and six doses of GM-CSF administration.

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The samples were allowed to stand and buffy coat containing WBCs was withdrawn, centrifuged and the pellet was washed three times with normal saline. The residual red cells were lysed by hypotonic lysis by treating the cells with ice cold distilled water for exactly 30 seconds and restoring tonicity using 0.6 M sodium chloride. Cells were pelleted by centrifugation and the lysis was repeated if found necessary. The preparation at this stage contained about 90% neutrophils. The viability of the cells was determined by Trypan Blue exclusion.

The activity of MPO was estimated by the method of Matheson et. al. (4). The total protein content of the lysate was determined by standard Lowry's method.

RESULTS AND DISCUSSION

All the patients in our study showed an increased activity of MPO to varying degrees. However, radiotherapy alone did not change the activity of enzyme. We also found a slight increase in the activity of the enzyme in most of the pre GM-

CSF samples compared to normal controls, which we attribute to the natural immune response. The test group showed a marked increase in the activity of the enzyme after GM-CSF administration (Table 1).

GM-CSF is a stimulator of neutrophils. Its effect on macrophage function has been studied (5). GM-CSF prolongs the life span of neutrophils, enhances their ability to kill antibody coated tumour cells; increases neutrophils adhesiveness and inhibits migration; thereby enhancing their activity on the target cells. GM-CSF can prime neutrophils so that they are more readily activated by formyl peptides, complement or leukotrienes resulting in the production of superoxide radicals which are handled *in vivo* by MPO and super oxide dismutase (5).

The mechanism of the beneficial effect of GM-CSF on radiotherapy induced mucositis may be a direct stimulatory effect of GM-CSF on the regeneration of the oral mucosa (6). GM-CSF may stimulate the oral mucosal cells to proliferate by enhancing transcription and translation of interleukin

Table 1. MPO activity (units/mg protein) in oral cancer patients with and without GM-CSF treatment and healthy controls.

Patients	Diagnosis	Sample - 1*	Sample - 2*	Sample - 3*
I Patient (55 Yrs)	Carcinoma of the anterior 2/3rd of the tongue.	0.13	0.15	0.59
II Patient (48 Yrs)	Cancer of the uvula, posterior part of the soft palate	0.12	0.13	0.48
III Patient (72 Yrs)	Carcionma of lip	0.11	0.13	0.53
IV Patient Control (55 Yrs)	Squamous cell carcinoma of the lip	0.05	0.06	NA
V Patient Control (42 Yrs)	Carcinoma of tongue	0.12	0.11	NA
VI Normal Control		0.05	NA	NA
VII Normal Control		0.07	NA	NA

Patients IV and V were treated with radiotherapy alone without GM-CSF.

* Sample 1: before radiotherapy; sample 2: between radiotherapy and before GM-CSF treatment; sample 3: after radiotherapy and GM-CSF treatment (see methods for details)

NA: not applicable.

- 1 (IL-1) (7). The protective effect of IL-1 on the lip mucosa against radiation damage has been reported earlier (7).

In the present study, the administration of GM-CSF was found to be clinically beneficial. In conclusion, our laboratory results show that the neutrophils were activated as evidenced by the increase in the enzyme (MPO) activity.

As GM-CSF is very expensive, we could include only 3 patients in this study. However, the results are promising for using GM-CSF as an adjuvant to radiotherapy and chemotherapy.

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REFERENCES

1. Chi, K.H., Chen, C.H., Chan, W.K., Chow, K.C., Chen, S.Y., Yen, S.H., Chao, J.Y., Chang, C.Y. and Chen, K.Y. (1995) Effect of Granulocyte Macrophage Colony Stimulating Factor on Oral mucositis in Head and Neck cancer patients after Cisplatin, Flourouracil and Leucovorin chemotherapy. *J. Clin. Oncol.* 13, 2620-2628.
2. Clark, S.C. and Kamen, R. (1987) The human heamatopoietic colony stimulating factors. *Science* 236, 1229-1237.
3. Groopman, J.E., Molina, J.M. and Scadden, D.T. (1989) Heamatopoietic growth factors: Biology and Clinical applications. *N. Engl. J. Med.* 321,1449-1459.
4. Matheson, N.R., Wong, P.S. and Travis, J. (1981) Isolation and properties of Human Neutrophil Myeloperoxidase. *Biochem.* 20, 325-330.
5. Jones, C.J. (1993) The effect of rh-GM-CSF on macrophage function. *Eur. J. Cancer.* 294, 510-513.
6. Roitt, I.M. (1992) *Encyclopedia of Immunology* Vol. 2. Academic Press, New York, USA p 630.
7. Dinarello, C.A. (1991) Interleukin 1 & interleukin 1 antagonism. *Blood* 77, 1627-1652.
8. Zaghloul, M.S., Dorie, M.J. and Kallman R.F. (1994) Interleukin 1 increases thymidine labeling index of normal tissue of mice but not the tumor. *Int. J. Radiat. Oncol. Biol. Phys.* 29, 805-811.