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Preservation of the lung function after thoracic irradiation: Role of transforming growth factor beta (TGF-β)

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TGF-β is considered as a predictor of pneumonitis in patients receiving radiotherapy. Plasma TGF-β levels were investigated in 27 consecutive patients with NSCLC stage III, who were treated with 60 Gy (2 Gy/day) radiotherapy with or without carboplatin. TGF-β was measured with a bioassay using mink lung epithelial cells transfected with a plasminogen activator inhibitor-1 promoter-luciferase construct (normal values (SD) 9.0 ng/ml (1.9)). Mean (SD) pretreatment TGF-β value was 55.8 ng/ml (33). Only 3 patients had normal values. TGF-β was not related to age, performance score, or weight loss. No relationship between pretreatment TGF-β levels and pneumonitis was observed. There was no influence of carboplatin or radiation field size on the incidence of pneumonitis nor on TGF-β levels during treatment. All 9 patients who developed pneumonitis (CTC criteria) after treatment had, during radiotherapy, a high persistent TGF-β levels relative to pretreatment levels. Patients with the same or lower TGF-β values relative to pretreatment values did not develop symptomatic lung injury. In conclusion, elevated TGF-β levels during radiotherapy may identify patients at high risk of developing pneumonitis after treatment. Furthermore, it can help in selection of those patients who might be candidates for dose escalation.

Key words: lung neoplasms-radiotherapy; transforming growth factor beta; radiation pneumonitis

Introduction

Radiation therapy plays a significant role in the treatment of lung carcinoma, Hodgkin's disease, breast cancer and other tumors involving thoracic region. Pulmonary tissue is one of the most critical dose limiting normal tissues involved in thoracic radiotherapy. The clinical sequelae of radiation lung injury usually start with the acute onset of radiation pneumonitis at 2 to 6 months after radiotherapy with the symptoms that range from cough, fever and dyspnea to death from respiratory failure. Depending on radiation dose and volume of the exposed lung, radiation pneumonitis may resolve without further changes or may progress to the stage of chronic pulmonary fibrosis significantly affecting the quality of patients' life. Identification of those patients that might be at high risk of developing pulmonary injury after thoracic irradiation as well as those in whom the dose of radiation can be safely increased would consequently lead to the preservation of the lung function and better tumor control.

There is considerable interest in transforming growth factor beta (TGF-β) as a mediator of normal tissue injury. It is suggested that the measurement of TGF-β in the plasma during chemotherapy/radiotherapy may be useful in predicting an individual patient's risk for developing late radiation-induced normal tissue injury.[1,2] The objective of this ongoing study is to investigate the utility of plasma TGF-β levels in identification of those patients at risk for the development of radiation-induced lung injury.

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Materials and methods

Plasma TGF-β levels were investigated in this ongoing study in 27 consecutive patients with NSCLC stage III, who were treated with 60 Gy (2 Gy/day) radiotherapy with or without carboplatin. All patients underwent history and physical examination, radiographic evaluation for staging purposes and had histologic confirmation of malignancy. Pulmonary function tests including total lung capacity (TLC), vital capacity (VC), forced expiratory volume per second (FEV_1) and diffusion corrected for alveolar volume (Kco) were obtained before and after the treatment. Plasma for TGF-β measurement was obtained prior to the beginning of radiotherapy and weekly during treatment. TGF-β was measured with bioassay using mink lung epithelial cells (MLEC) transfected with a plasminogen activator inhibitor-1 promoter-luciferase construct (normal values (SD) 9.0 ng/ml (1.9)). This bioassay is based on the ability of TGF-β to induce plasminogen activator inhibitor-1 (PAI-1) expression. MLEC were stably transfected with an expression construct containing a truncated PAI-1 promoter fused to the firefly luciferase reporter gene. Binding of TGF-β to the transfectants results in a dose-dependent increase in luciferase activity in the cell lysate assay was described by Abe *et al.*[3] and recently modified in our laboratory.[4] The endpoint of the study was the development of symptomatic radiation pneumonitis defined according to the National Cancer Institute Common Toxicity Criteria (CTC).

Results

Of the 27 NSCLC patients, 14 had squamous cell, 7 adenocarcinoma and 6 large cell carcinoma. Thirteen patients had stage IIIA and 14 IIIB NSCLC. All patients were treated with curative intent, 17 with radiotherapy and carboplatin, 10 with radiotherapy alone.

Mean (SD) pretreatment TGF-β value was 55.8 ng/ml (33). Only 3 patients (11%) had normal pretreatment values. TGF-β was not related to age, performance score, or weight loss. There was no correlation between the pretreatment TGF-β values and either the incidence or severity of pneumonitis. There was no influence of carboplatin or radiation field size on the incidence of pneumonitis nor on TGF-β levels during treatment. Pretreatment lung function values in % predicted of TLC, VC, FEV_1 and Kco (SD) were 87 (16), 86 (19), 66 (18), 116 (25), resp. Posttreatment values of TLC, VC, FEV_1 and Kco (n=25) were 83 (16), 83 (13), 65 (13), 105 (25). Changes in pulmonary function tests did not correlate with the development of pneumonitis or plasma TGF-β levels. The patients were divided into two groups according to whether or not they developed symptoms of radiation induced pulmonary injury (CTC criteria). Nine of the 27 patients developed pneumonitis. The patients who developed pneumonitis had high persistent TGF-β levels throughout the course of treatment (TGF-β ratio > 1), whereas the TGF-β levels in patients who did not develop pneumonitis stagnated or turned to normal (TGF-β ratio =< 1). This ratio is defined as the ratio between TGF-β at each week of radiotherapy normalized and the pretreatment TGF-β level. The difference in plasma TGF-β levels between the patients with and without radiation-induced pneumonitis become significant four weeks after the beginning of radiotherapy treatment (p=0.028).

Discussion

The involvement of cytokines as mediators of normal tissue injury and repair following the treatment with radiation and/or chemotherapy is currently a topic of intense laboratory and clinical research. Among the many cytokines that have so far been recognized, transforming growth factor beta (TGF-β) is of particular interest. TGF-β is a multifunctional regulator of cell growth and differentiation which stimulates connective tissue formation and decreases collagen degradation resulting in fibrosis.[5] Recent studies have reported locally produced TGF-β to be involved in the promotion of radiation-induced fibrosis in a number of tissue types.[6-10] The role of TGF-β in radiation-induced pulmonary fibrosis is, however less certain.

Finkelstein *et al.*[11] found very early fluctuations in whole lung TGF-β gene expression at 1 and 14 days after irradiation in mice. More recent results from the same group using radiation fibrosis-prone mice indicated elevated TGF-β levels in the lung that persisted at least until 8 weeks after irradiation.[12] Similar increase in mouse lung TGF-β levels have been observed following the treatment with bleomycine, or cyclophosphamide. The later phenomenon is especially pertinent to the potential enhancement of pulmonary toxicity by the combination of chemotherapy with thoracic irradiation.

An association between circulating levels of TGF-β and the incidence of pulmonary complications was first reported in patients receiving high-dose chemotherapy in the setting of autologous bone marrow transplantation for advanced breast cancer.[2] The same authors later reported preliminary findings which indicated that plasma TGF-β levels measured during radiotherapy for lung cancer may be useful in identifying patients who will or will not go on to develop symptomatic radiation pneumonitis.[1] The present study is a further confirmation that measurement of TGF-β during radiotherapy treatment may be a useful tool for identifying patients at risk for the development of radiation pneumonitis. Our data are also suggesting that other physical parameters, such as volume of irradiated lung or changes in lung function do not correlate with the risk of developing pneumonitis. This emphasizes the importance of TGF-β in the development of radiation induced lung injury. Understanding that role might further help in preservation of the lung function. Recently, it has hypothesized that normal tissue injury is not only mediated by the local production of TGF-β but also influenced by elevated circulatory level of TGF-β produced by the tumor.[13] The evidences exist that newly diagnosed breast, liver and, as our results show, lung cancer patients have elevated pre-treatment plasma TGF-β levels.[14,15] However, it is difficult to resolve what is the contribution of a normal tissue injury in TGF-β production during radiation therapy and what is produced by existing tumor.

In conclusion, elevated TGF-β levels during radiotherapy treatment may identify patients who develop pneumonitis after treatment. Further experimental and clinical investigations on the role of TGF-β and other cytokines in chemotherapy- and radiotherapy-induced injury to the lung are certainly necessary.

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