Radiotherapy of tumors in the chest and neck regions may have serious pulmonary side-effects. It is well known that inflammation is an essential manifestation of radiation-induced injury. This can heal spontaneously, by specific treatment, or it may progress to more intensive inflammation up to irreversible pulmonary fibrosis. To prevent such complications, it would be useful to have a simple non-invasive and sensitive method for monitoring the course of airway and lung post-irradiation inflammation. This study is devoted to search for such a method. We supposed that cough response intensity (CRI) could be one of the methods, which we are looking for. Guinea pigs (Trik strain, n=32) were used in the study. Animals were divided into two subgroups. Animals of a non-untreated (NT) group (n=14; M=7, F=7) were submitted to sham chest irradiation. The animals of a treated (XRT) group (n=18; M=9, F=9) were exposed to a single dose of gamma rays. Cough was provoked by exposure of animals to citric acid aerosol (CA) in gradually increasing concentrations (0.05 – 1.6M). CRI testing was performed two days before sham/real chest irradiation, than on 1\textsuperscript{st}, 3\textsuperscript{rd}, 10\textsuperscript{th}, 15\textsuperscript{th}, 21\textsuperscript{st}, and 28\textsuperscript{th} days following the day of irradiation. CRI was quantified in each animal by counting the number of coughs induced by all used concentration of CA. We found a significant increase of CRI in the animals of XRT group on 10\textsuperscript{th} and 21\textsuperscript{st} day compared with the NT animals. An increase of CRI also was found inside the XRT group on the 10\textsuperscript{th} day after irradiation compared with the pre-irradiation value of CRI.

Key words: citric acid-induced cough, guinea pig, thoracic irradiation

INTRODUCTION

Radiation therapy to treat cancer is inevitably accompanied by untoward exposure of normal tissues. This results in radiation injury which can
substantially decrease positive effects obtained by radiotherapy. The lung is one of the most radiosensitive organs, yet is frequently irradiated as part of treatment programmes for cancers of the lung and surrounding organs (esophagus, breast, lymphatic system, etc.). The clinical phase of radiation injury in the lung becomes apparent after a delay of 1-3 months, and it manifests as cough, dyspnea, fever, and chest pain. The cause of the mentioned symptoms and signs is mainly radiation pneumonitis (RP) (1). Bronchoscopic microbiopsy with histological examination can be used for detection of RP after 4-6 weeks, but this method is too invasive to be repeatedly used for monitoring of the time course of RP development. Traditionally, radiographic methods (high resolution computed tomography – HRCT, chest radiography) are used for diagnosis of RP but these methods can detect changes only several weeks after the onset of radiotherapy. It would be useful to have a simple non-invasive and sensitive method for monitoring the course of airway and lung post-irradiation inflammation.

Data from animal and human studies indicate that vascular injury and activation of coagulation cascade, creation of cellular adhesion molecules, production of proinflammatory and profibrotic cytokines, and oxidative stress, all seem to play a vital role in the development of RP (2-5). It also is known that the airway inflammation can sensitize the cough nerve-endings in the airway mucosa (6), which can present as dry cough. Therefore, we hypothesized that the radiation-induced airway and lung inflammation could influence the cough response intensity (CRI) in experimental animals and humans.

In our previous study in humans, we have shown that radiation exposure of airways and lung in patients undergoing radiotherapy of cancer in the thorax and neck regions led to elevation of CRI two weeks after the onset of radiotherapy (7). In addition, in a pilot experimental study done in our laboratory, we have shown an increased CRI in guinea pigs on the 6th day after thoracic irradiation by a single 10 Gy dose (8). In the present study, we would like to extend those findings by increasing the dose of irradiation, the number of animals used, and by extending the observation period during which the CRI will be measured. Therefore, the major aim of our study was to find out whether chest irradiation with a dose of 12 Gy will change CRI in a similar way as irradiation with 10 Gy, and to describe the CRI changes during one month following the irradiation of the chest.

MATERIAL AND METHODS

Animals

The study was approved by the Ethics Committee of the Jessenius Faculty of Medicine in accordance with the Helsinki Declaration of 1975, as revised in 1983. Thirty-two (16 M and 16 F) adult Trik strain guinea pigs (Cavia porcellus), weighing 250-350 g were used in the study. The animals were adapted to conditions present in the animal house. They were housed in cages at a mean temperature of 24°C for 1 week after they were transported from breeding setting (Dobra
Irradiation

The animals were divided into two subgroups: without irradiation (no treatment – NT group) consisting of 14 animals (7 M, 7 F), these animals underwent sham irradiation (control group), and with irradiation (experimental XRT group), which underwent thoracic irradiation with a dose of 12 Gy consisting of 18 animals (9 M and 9 F). To prevent any movements of animals during real/sham irradiation they were anesthetized with ketamine (100 mg/kg, i.p.) and immobilized on a special Perspex pad. Radiation dose was delivered with a single ventral-dorsal field using \(^{60}\)Co gamma rays to the whole thorax. The irradiation characteristics were as follows: beam energy: 1.3 MV-photons; dose-rate: 1.6 Gy/min; source - surface distance (SSD): 0.8 m; size of the radiation field: 5x4.5 cm. Untreated, sham-irradiated animals were handled exactly in the same way, except that they did not undergo irradiation.

Cough response

Citric acid-induced cough was recorded at seven different time points – two days before, and then on the 1\(^{st}\), 3\(^{rd}\) (the XRT group only), 10\(^{th}\), 15\(^{th}\), 21\(^{st}\), and 28\(^{th}\) day following the irradiation day. At the end of the experimental protocol, the animals were sacrificed by an overdose of the anesthetic.

For quantification of cough response intensity (CRI), we used a method described previously (8). Each conscious guinea pig was placed in an airtight double-chamber, transparent plastic body plethysmographic box (type 855, Hugo Sachs Electronic, Germany) and was exposed first to a nebulized control solution (0.9% saline) and then to doubling concentrations of citric acid (CA) (from 0.05 to 1.6 M, Lachema). Each concentration of nebulized CA was inhaled for 30 s, with a 1-min interval between exposures. Aerosol was produced a jet nebulizer (Pariprovocation test I, Pari Starneberg, Germany; output 5 l/min, particle mass median diameter 1.2 \(\mu\)m). The citric acid aerosol was delivered to the head chamber of the plethysmographic box. A Fleish head was connected to the head chamber. Microphone for recording of cough sounds was placed in the roof of the head chamber and connected to a tape recorder. The number of coughs was counted during a 30 s inhalation of each CA concentration and during the subsequent 1 min observation time.

The number of coughs was counted by a trained observer using three different methods to ensure that only coughs were counted and that sneezes and augmented breaths were excluded. The three methods were as follows: i) observation by an observer trained to differentiate between coughs and sneezes according to changes in animal body posture and movements (spaying of the front feet and forward stretching of the neck) and the characteristic opening of the mouth associated with cough, ii) by pneumotachograph - coughs were detected as a transient change in the airflow (a rapid inspiration followed by rapid expiration), and iii) by sound - the characteristic sound of guinea pig cough was distinguished using a self developed software system. Differentiation between cough and sneeze was based on a spectral analysis of respective sounds (digitized at a sampling frequency of 11 025 Hz). As was shown previously, cough sound differs from sneeze sound by a shorter duration, a lower frequency of maximal spectral power peak, and a steeper increase of sound intensity at the beginning of sound (9, 10). The intensity of cough response was expressed as the sum of all coughs produced by the animal during its exposition to all concentration of citric acid.

Data analysis

Statistical analysis was performed using a SYSTAT 10 (SSI, Richmond, CA, USA) statistical package. Due to non-gaussian distribution of variables (confirmed by Lilliefors test),
nonparametric tests were used. Between group differences in CRI (NT vs. XRT group) were analysed using Mann-Whitney U test. The overall effect of sham/real irradiation on CRI within each group was analyzed by nonparametric Friedman’s test. In case of an overall significant effect, Wilcoxon’s signed-rank test was used as a post hoc test for assessing differences in CRI on all days after treatment compared with its value before treatment. Differences were considered significant at \( P \leq 0.05 \).

**RESULTS**

**Effect of thoracic irradiation on cough response intensity – between-group comparison**

Before irradiation, no significant difference in CRI between the two groups was found (\( P=0.97 \)). After irradiation, significant differences between the NT and XRT groups on the 10\(^{th}\) and 21\(^{st}\) day were found (\( P<0.05 \)), with a higher count of coughs in the XRT group. There were no between-group differences on the 15\(^{th}\) and 28\(^{th}\) day after sham/real irradiation (\( P=0.47 \) and \( P=0.11 \), respectively) (Fig. 1).

**Effect of thoracic irradiation on cough response intensity – within group comparison**

Friedman’s test showed significant differences in CRI during the observed period only in the XRT group (NT group: \( P=0.52 \), XRT group: \( P=0.01 \)). An analysis by Wilcoxon’s test, comparing values of CRI before and after irradiation, revealed significantly higher values of CRI on the 10\(^{th}\) day after irradiation in the XRT group (\( P=0.04 \)). In addition, a tendency to higher CRI also was found on the 3\(^{rd}\) day after irradiation in this group (\( P=0.06 \)) (Fig. 2).

**DISCUSSION**

The major findings of this study were that CRI increased in the irradiated animals on the 10\(^{th}\) day after thoracic irradiation compared with the pre-irradiation period, and that CRI was higher in the irradiated group on the 10\(^{th}\) and 21\(^{th}\) day after radiation exposure compared with control animals.

Early detection of RP is very important for its adequate treatment. Traditionally, radiographic methods (high resolution computed tomography – HRCT, chest radiography) are used for the diagnosis of RP, but these methods can detect changes only several weeks after the onset of radiotherapy and cannot be used as a monitoring method, because of their possible side effects and high cost. Although histopathologic changes accompanying RP can be detected earlier, the sample taking is too invasive to be repeatedly used. Several other methods for early detection of RP, including cytokine detection (e.g., interleukins, tumor necrosis factor-TNF, etc.) in the serum or bronchoalveolar
Fig. 1. Box plot of the effects of thoracic irradiation with a single dose of 12 Gy on cough response intensity on 1st (D1), 10th (D10), 15th (D15), 21st (D21), and 28th (D28) day after irradiation in the XRT (with irradiation) and NT (no treatment-sham irradiation) groups. The length of each box shows the range within which the central 50% of the values fall, with the box edges at the first and third quartiles. The central horizontal line in a box marks the median. Open circles and asterisks indicate outliers. Significant between-group differences (XRT vs. NT) are indicated (#P≤0.05, Mann-Whitney U-test).

Fig. 2. Box plot of the effects of thoracic irradiation on cough response (expressed as a number of coughs) on 1st (D1), 3rd (D3), 10th (D10), 15th (D15), 21st (D21), and 28th (D28) day after irradiation in the XRT group (exposed to 12 Gy irradiation). Boxes comprise interquartile range of values and circles and asterisks indicate outliers. Significant differences between baseline (before) and D10 value is indicated (#P≤0.05).
lavage fluid (5, 11, 12), and detection of levels of blood surfactant fractions (13) were introduced into experimental studies. The examination of induced sputum or exhaled NO are another potential methods of early detection of radiation injury (14). Various clinical and experimental studies have shown that determination of Clara cells protein 16 (CC 16) is a new sensitive marker of lung epithelial barrier damage (15). Using these methods for monitoring of post-irradiation inflammatory processes in the airway has a good perspective, but their application is limited only to research purposes due to the cost and complexity. Therefore, there is a need to find another simple, sensitive, specific enough, and practicable noninvasive method for detection and monitoring the radiation inflammation.

One of the potentially useful methods for detection of early phase of RP could be an assessment of cough response to tussigenic agents. It is very well known that any type of airway inflammation is able to change the functions of airway nerve endings mediating cough reflex (16, 17). An array of inflammatory mediators released from damaged airway and lung epithelial cells have a potential to increase or decrease sensitivity of airway cough receptors. Recently, it has been shown that a cough reflex sensitivity test can be even a more sensitive marker of airway inflammation than routine functional lung tests (18, 19).

In our previous study, we have shown that radiation exposure of airways and lungs in patients undergoing radiotherapy of cancer localized to the thorax and neck regions led to an elevation of cough response two weeks after the onset of radiotherapy, compared with a group of breast cancer patients in whom a more superficial irradiation was used (7). These findings prompted us to design an experimental study in guinea pigs to gain an insight into the mentioned phenomenon. Twelve animals were irradiated in the thoracic region with a single dose of 10 Gy and we have observed an increase in CRI on the 6th day after irradiation compared with the control group (8). In the present study, we extended those findings using a bigger group of animals and a prolonged observation period. We found that cough reactivity was elevated on the 10th and 21st day after a single 12 Gy irradiation dose. We thus confirmed the previous results, and it seems clear that irradiation of the thorax leads to changes of cough reactivity. It is difficult to explain why the increase in CRI is present only during selective days following irradiation. Some experimental results (5) have demonstrated that there is time-dependent release of different types of proinflammatory mediators by airway and alveolar cells after lung radiation injury, which can be responsible for this phenomenon. To validate the CRI as an early marker of lung radiation injury, suitable for monitoring of post-irradiation airway and lung inflammation, we have to detect simultaneously additional inflammatory markers as mentioned above.

We conclude that changes in cough response intensity have a potential to become one of the markers able to reveal early functional changes induced by irradiation in the lungs. Further studies on sensitivity and specificity of this
method and its relation to other (e.g., biochemical) markers of irradiation lung damage should be performed.

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**REFERENCES**


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