3 SUMMARY

Bioindicators of inflammation, oxidative and nitrossative stress in Wistar rats after whole-body and lung irradiation

Introduction

Radiation pneumonitis and fibrosis limit the intensity of radiotherapy, increase morbidity and worsen quality of life of patients with lung and breast cancer or Hodgkin's disease. Radiation pneumonitis is an acute inflammatory reaction that resolves either spontaneously or after treatment with corticosteroids. Free radicals play an important role in its etiology. They cause dose-dependent induction of lipoperoxidation, DNA damage, activation of proapoptotic events and changes in activity of enzymes. The inflammatory reaction initiated by free radicals affects primarily epithelial and inflammatory cells, which release a number of cytokines (TNF-α, IL-1, IL-6, TGF-β, etc.). Radiation fibrosis of lung develops as a late consequence of radiotherapy (> 3 months). It can lead to chronic respiratory failure and death. Macroscopic findings include fibroblast proliferation, collagen deposition and destruction of lung parenchyma.

Aims and methodology

This work was focused on the changes in the arginine-nitric oxide metabolic pathway induced in the airways, lung and other parts of the body by ionizing gamma radiation in the early phase (within 72 h) as well as in the phase of radiation pneumonitis (7 weeks) following exposure to whole-body and local chest irradiation of Wistar rats. The value was evaluated of the oxidative and nitrossative stress biomarkers for radiation biodosimetry and prediction of the onset of radiation pneumonitis. The concentration of exhaled nitric oxide (eNO) was measured noninvasively using chemiluminiscence. Arginine, nitrites/nitrates (NOx) and malondialdehyde (MDA) were determined by high performance liquid chromatography in the plasma, bronchoalveolar lavage fluid (BAL) and lung tissue homogenate. Expression of an inducible form of nitric oxide synthase (iNOS) was studied in samples of lung tissue by Western blotting and gene expression of iNOS, arginases and arginine transporters (CAT) was assessed by RT-PCR. The presence of radiation pneumonitis was verified histochemically (airiness of the lung and neutrophil count in the lung tissue). Moreover, radioprotective effects of selected antioxidants and inhibitors of NOS [L-N-nitroarginine methyl ester (L-NAME), aminoguanidine (AG), acetyl-L-carnitine (ALC)] were evaluated.

Results

In the interval of 72 h following whole-body irradiation (2-50 Gy), the changes in the concentrations of NO and other nitrossative stress markers in the airways were either modest or absent. The iNOS protein was not detected in the lungs and its expression was comparable in the liver of irradiated rats and non-irradiated controls. Concentrations of eNO exhaled by irradiated and control rats were comparable during both the early phase after whole-body exposure (72 h) and the radiation pneumonitis phase following chest irradiation. Whole-body irradiation with 2-10 Gy caused a dose-dependent increase in plasma NOx up to the maximum level four times above the control value ($P<0.001$). Single injection with L-NAME and five-day pretreatment of rats with ALC before whole-body irradiation (8 Gy) improved the survival of rats in the period of 30 days after exposure from 35% to 75% ($P<0.005$). In irradiated rats, L-NAME suppressed plasma NOx while ALC prevented the increase of plasma MDA. Seven weeks following thorax irradiation (20 Gy), an increased concentration of protein (5-fold, $P<0.01$) and MDA (1.8-fold, $P<0.05$) was found in BAL of rats with histologically proven radiation pneumonitis. Furthermore, there was an increased gene expression of arginase I and CAT transporters 1, 2 and 3, respectively, in the lung of irradiated animals ($P<0.05$). All compounds under the study increased the airiness of the lung and/or reduced the inflammatory infiltrate seven weeks after thoracic irradiation (15 Gy). In addition, ALC significantly improved survival of rats from 30% to 80% ($P<0.05$) and decreased the expression of arginase I, CAT1 and CAT3 in the lung tissue of irradiated rats ($P<0.05$).

Conclusion

The results show that noninvasive measurement of eNO cannot be used as a biodosimetric indicator or predictor of radiation pneumonitis in rats. Changes in the metabolic pathway of L-arginine-NO caused by ionizing radiation in the airways are mild. L-NAME and ALC exert protective effects against whole-body irradiation of rats. The results of the seven-week assessment following thoracic irradiation with 15 Gy document that ALC is the best radioprotector among the compounds under study. The reduced expression of arginase I and arginine transporters CAT1 and CAT3 in the lung of the ALC-pretreated and irradiated rats might indicate that the compound reduces the intensity of radiation pneumonitis thereby decreasing the demands on the pulmonary availability of arginine and polyamines necessary for its healing.

Key words: exhaled nitric oxide, ionizing radiation, radiation pneumonitis, radioprotection