Inhibitory effect of ionizing radiation on cellular adhesion of lymphocytes to endothelial cells under dynamic conditions*

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Introduction

Chronic inflammatory diseases are efficiently treated by irradiation with low doses of photons (low dose radiotherapy) or α-particles (radon treatment), but the cellular and molecular background remains widely unknown [1,2]. Adhesion of PBMC on the endothelial cell (EC) wall of the blood vessels is one of the initial steps in the inflammation cascade to recruit immune cells to inflamed tissue. An inhibitory effect of photon irradiation on the adhesion process is discussed as part of the anti-inflammatory mechanisms of low dose radiotherapy [3,4]. In our previously reported experiments EC were all cultured under static conditions, whereas in vivo EC are exposed to constant shear stress of the blood stream. Therefore the goal of the present study was to investigate possible changes in the adhesion of peripheral blood lymphocytes (PBL) to primary EC under dynamic cultivation conditions and after irradiation with X-rays or carbon ions. Because of physical differences in the energy deposition between photons and α-particles, both radiation qualities were compared with respect to their anti-inflammatory effectiveness. Carbon ions with similar LET values were used as a surrogate for α-particles.

Materials and Methods

A flow-chamber system was built, based on a publication by Freyberg et al. [5]. Human dermal microvascular endothelial cells (HMVEC) were used for the experiments. They were cultivated for 72h prior to exposure to X-rays (250kV, 16mA) or carbon ions (LET: 168keV/µm). After irradiation EC were stimulated with TNF-α (1ng/ml) and incubated for 24h under laminar flow conditions. Afterwards the complete medium was exchanged by medium w/o TNF-α supplementation. Afterwards stained PBL were streamed over the EC monolayer for 30min. The unbound PBL were removed by washing the samples with PBS and afterwards EC were fixed with paraformaldehyde. Analysis was performed using a fluorescent microscope. As a comparison all experiments were performed as well under static conditions.

Results and Conclusion

The adhesion of PBL on HMVEC was measured after exposure to a low (0.5Gy) or a high dose of photons or carbon ions (2 or 6Gy, respectively). The results are shown in figure 1. The relative adhesion indicates the amount of attached PBL normalized on the positive controls (unirradiated and stimulated) which were set as 100%. As can be seen from figure 1, the relative adhesion of the unstimulated controls is only about 30% compared to the positive controls. After irradiation of HMVEC with a low dose of either photons or carbon ions, the adhesion of PBL was remarkably reduced (to about 30%). This corresponds roughly to the level of the unstimulated controls. However, after irradiation with a high dose a reduced adhesion was only observed for carbon ions (2Gy) but not for X-rays. The dose response remains to be investigated in more detail. By these results we can show a clear inhibitory effect of sparsely- and densely ionizing radiation on adhesion of PBL to EC.

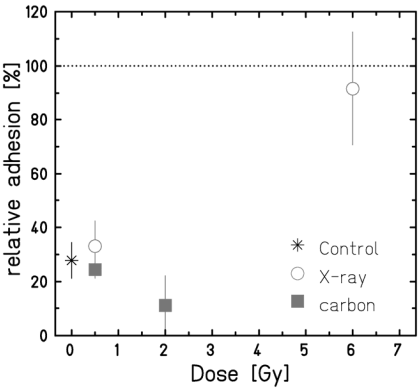

Figure 1: *Influence of irradiation with X-rays or carbon ions (LET: 168keV/µm) on adhesion related interaction of TNF-α stimulated ECs and PBL under dynamic conditions (100% adhesion corresponds to unirradiated, TNF-α stimulated EC).* (X-ray: N=2-4, n=5-9; carbon ions: N=1, n=2)

Although we did not find a specific low dose effect in previously performed experiments under static conditions, we observed a high effectiveness of low doses for both radiation qualities with primary EC under dynamic conditions [6]. In accordance to our results a low dose effect was also observed in human genetically engineered EC or murine endothelioma cells [3,4,7] as well *in vivo* for gastrointestinal venules of mice [8]. This points to complex molecular interactions promoting or inhibiting the adhesion processes which are influenced by sheer forces of the bloodstream.

References

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