EXCLI Journal 2003;2:52-57 – ISSN 1611-2156

received: 27. October 2003, accepted: 2. November 2003, published: 6. November 2003

Original article:

Effect of Cobalt-60 Irradiation on Bradykinin B2 Receptor Expression on Human HF-15 Cells

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ABSTRACT

Bradykinin is a key mediator of pain and inflammation. Although radiotherapy has proven to be beneficial in the treatment of inflammatory disorders, the effect of irradiation on the bradykinin pathway in human cells has not been evaluated yet. Therefore, the aim of the study was to establish a human cell culture system and to analyze bradykinin B2 receptor expression in response to different doses of gamma-ray exposure. Cultured human foreskin fibroblasts (HF15) were irradiated with 0.5 Gy, 2.0 Gy, 5.0 Gy and 10.0 Gy single doses using a Cobalt 60 radiation source. Before treatment (0h) as well as 6, 24, and 48 hours after radiation the bradykinin receptor surface density was quantified by a ligand binding assay using radioactive [^{3}H]bradykinin. A dose and time dependant expression of the bradykinin B2 receptor was observed. Initially, higher doses (2 and 10 Gy) induced a fast upregulation of the receptor, followed by long lasting downregulation compared to baseline levels. In contrast the lowest dose (0.5 Gy) induced a fast down regulation of the receptor. After 24 h and 48 h the levels increased again but remained below baseline levels. A dose- and time-dependant change in bradykinin B2 receptor expression on HF-15-cells in response to irradiation was demonstrated. The results may imply radio-biological explanations for the beneficial effect of radiotherapy in benign inflammatory diseases.

Keywords: Bradykinin, receptor expression, B2-receptor, inflammation, radiotherapy

INTRODUCTION

The nonapeptide bradykinin (NH_2-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg-COOH), a prototypic member of the kinin family, is a key mediator in the initiation and maintenance of inflammatory processes (Kaplan et al., 2002). It is locally released on the surface of target cells due to limited proteolysis of their parental molecules, kininogens, by the kallikreins (Blaukat et al., 2002) Its effects are transmitted via activation of the bradykinin B2 receptor (Regoli and Barabe, 1988) potentially leading to the formation of pro-inflammatory cytokines (e.g. IL-1 and TNF-α) as well as second generation mediators like platelet activating factor, leukotrienes, prostaglandins, and nitric oxide (Uknis et al., 2001; Tiffany and Burch, 1989, Bareis et al., 1983). In addition, bradykinin is able to either excite nociceptors directly or to sensitize them to mechanical or heat stimulation (Neugebauer et al., 1989; Kanaka

et al., 1985; Lang et al., 1990). In pathological states the multiplicity of biological effects can result in the cardinal signs of inflammation, namely tumor (edema), rubor, calor and dolor (Blaukat, 2003; Cassim et al., 2002). Consequently the bradykinin pathway is considered to be involved in nearly all acute and chronic inflammatory diseases. Especially in inflamed joint diseases the importance of bradykinin and its correspondent B2 receptor is well documented (Cassim et al., 2002; Griesbacher et al., 2000, Stewart et al., 1999). The injection of bradykinin induced acute arthritis in dogs (Lerner et al., 1987) and elevated kinin levels were found in patients with rheumatoid osteoarthritis (Bond et al., 1997, Jasani et al., 1989) The use of synthetic B2 receptor antagonists can effectively inhibit the release of pro-inflammatory mediators and clinical symptoms (Mello et al., 2002; Uknis et al., 2001; Burgess et al, 2000).

Since the introduction of radiotherapy in clinical medicine in 1896 and during the first decades of the 20th century the treatment of benign inflammatory disorders with radiotherapy was a main domain of radiotherapists (Sokoloff, 1898; Leer et al., 1998). However, after publications on potential induction of malignancies (Cour-Brown et al., 1965; Lindelöf et al., 1986; van Daal et al., 1985) this option was discredited and consequently restricted to few indications, particularly in the Anglo-American countries (Leer et al., 1998; Seegenschmidt et al., 2000). With the introduction of modern techniques, optimized schedules and smaller fields, the anti-inflammatory and analgesic effects of low-dose irradiation has again become a focus in clinical and research (Micke et al., 2002; Rödel et al; 2002). Although the biological mechanisms behind clinical improvement are poorly known, some recent studies revealed a significant modulation of important pro-inflammatory molecules including cytokines and adhesion molecules (Rödel et al. 2002, Hildebrandt et al., 2002; 2000, 1998).

Since there are no data available that elucidate the effects of low dose ionizing radiation on the bradykinin pathways, an in-vitro model of cultured human fibroblasts was established and the expression of the bradykinin B2 receptor after irradiation with different doses at successive time points was analyzed.

METHODS

Cell Culture

HF-15 human foreskin fibroblasts were grown to confluence in Dulbecco's modified Eagle's medium containing 10 % fetal calf serum, 1 % streptomycin-penicillin and 1 % L-glutamine for 2-3 weeks and used at passages 15-20 (Blaukat et al., 1996).

Irradiation

Cultured cells were irradiated using the gamma-rays (ca. 1.2 MeV) of a Cobalt 60 treatment unit (Fa. Phillips, Germany) with single doses of 0.5 Gy, 2.0 Gy, 5.0 Gy and 10.0 Gy. In order to compensate the build-up effect 5 mm water equivalent bolus material was used. Subsequently the cells were cultured for various time intervals (0, 6, 24, 48 hours). All experiments were performed as triplicates.

Ligand-binding-assay

At time points 0, 6, 24, and 48 hours after radiation the bradykinin receptor density on the cell surface was measured. Membrane bradykinin B2 receptor expression was quantified by a ligand binding assay using radioactive [3H]bradykinin according to established procedures (Blaukat et al. 1996). In brief, cells were harvested in 20 mM PIPES, pH 6.8, 2 mM Bactitracinand collected by centrifugation at 310,000 g for 30 min at 4 °C and used for ligand binding assays. Binding assays were performed by incubation with 5 nM [^{3}H]bradykinin, 2 mM bacitracin in the same medium in the absence (total binding) or in the presence (nonspecific binding) of 5 μM unlabeled bradykinin for 90 min at 4 C. The unbound ligand was removed by filtration trough flass fiber filters and 3x washes with 14 % isopropanol. Filter

and thus membrane bound [^{3}H]bradykinin was quantified in a β-counter.

Data analysis

All experiments were performed as triplicates. Differences were calculated using the paired T-test. P-values below 0.05 were considered to be significant.

RESULTS

Time-dependent kinetic

Bradykinin B2 receptor expression varied depending on the applied dose (figure 1). When high doses of radiation were used (10 Gy, 2 Gy) the B2 receptor expression increased significantly to 136% and 123% from baseline (6h, $p < 0.05$). In contrast, 5 Gy irradiation showed no significant change after 6h (76 %), whereas low dose irradiation with 0.5 Gy induced a significant decrease to 29%. The expression curves of the higher dose groups (10, 5, 2 Gy) declined after 24 h and where significantly lower after 48 h compared to baseline (62 %, 65 %, and 65 %, respectively, $p < 0.05$ 0h versus 48h). In low dose (0.5 Gy) irradiated cells the B2 receptor was unregulated at the time point 24 h and 48 h, but remained slightly but significantly below the baseline measurements ($p < 0.05$).

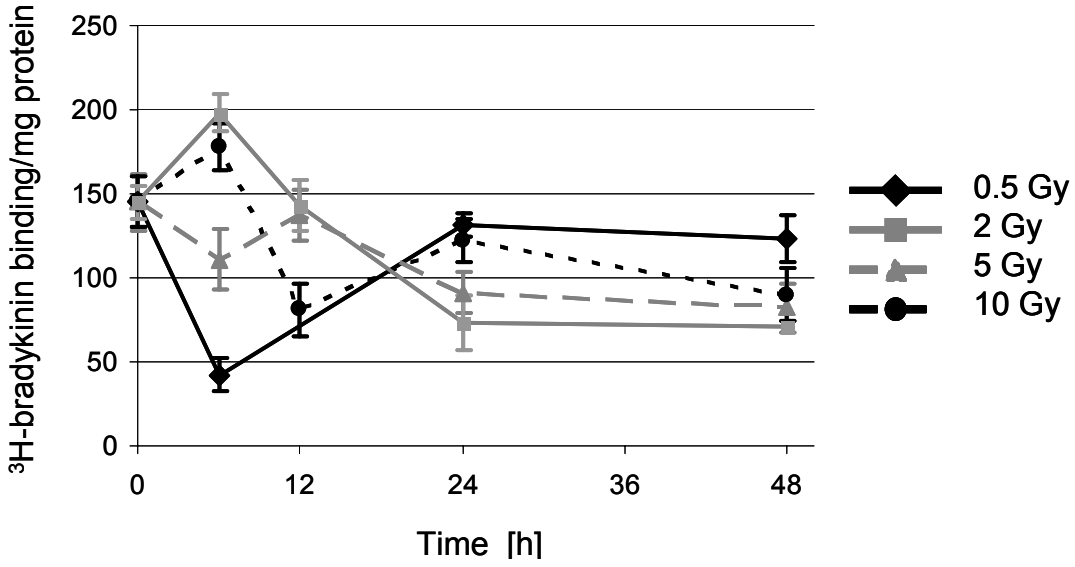

Figure 1: Time course of bradykinin B2 receptor expression. 3H-bradykinin binding to human HF15 cells were measured before and after 6, 12, 24 and 48 h after radiation. Data points present average and standard deviation of three experiments.

Dose-dependent kinetic

The most pronounced effect occurred after 6 hours (figure 2). 0.5 Gy showed a significant increase, whereas 2 and 10 Gy showed a significant increase in receptor expression. At time point 48 h there was a consistent decrease of B2 receptor expression in cell samples treated with higher does of irradiation (2, 5, 10 Gy). The low dose treated cells (0.5 Gy) responded only with a modest non-significant decrease after 48 h.

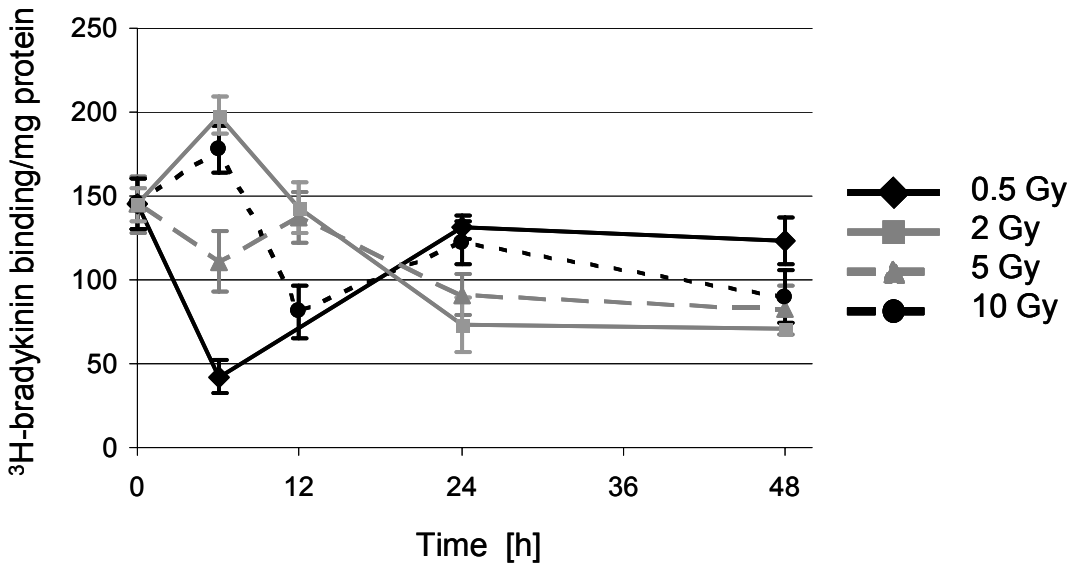

Figure 2: Dose response of bradykinin B2 receptor expression. ^{3}H-bradykinin binding to human HF15 cells were measured after the application of 0.5, 1.0, 2.0 and 5.0 Gy. Data points present average and standard deviation of three experiments.

DISCUSSION

The peptide hormone bradykinin and its corresponding B2 receptor are potent mediators of pain and inflammation (Kaplan et al., 2002). In particular in chronic inflammatory joint diseases increased levels of ligand or/and receptors were observed (Bond et al., 1997; Burgess et al., 2000; Cassim et al., 2002). This pilot study analyzed for the first time the influence of ^{60}Co γ-rays on bradykinin B2 receptor expression. In order to establish a well defined *in vitro* system without artificial transfection, the human fibroblast cell line HF15 which expresses the B2 receptor endogenously was chosen. The presented results indicate a dose and time dependent cell response to ^{60}Co γ irradiation in respect to Bradykinin B2 receptor expression. Low dose irradiation with 0.5 Gy resulted in a marked decrease in receptor expression after a short time, which may be interpreted as marked inhibition of inflammatory response. Higher dose showed a tendency to increase expression in short term, which was followed by a time-dependent decrease well below baseline levels.

The results correspond to molecular and clinical effects of low dose treatment in inflamed joint diseases. Low dose irradiation (total doses 2.5 Gy and 5 Gy with single doses of 0.5 resp. 1.0 Gy) lead to a long lasting reduction of the arthritis score in mice with adjuvant induced arthritis (Hildebrandt et al., 2000). The clinical effects went along with a significant reduction of iNOS (inducible nitric oxide synthetase), a pro-inflammatory mediator, that can be induced by B2 receptor stimulation. In an arthritis rabbit model irradiation significantly reduced proliferation of synovial lining fibroblasts, production of synovial fluid and swelling of the knee joint (Budras et al., 1986). These anti-rheumatic effects could be reproduced in a variety of animal models with different types of inducible arthritis (Schurmann et al., 1981; Trott et al., 1995; Fischer et al., 1994, 1998). Interestingly, the application of the synthetic B2 receptor antagonist (HOE 140) revealed comparable - but faster - reduction of inflammatory markers like iNOS or Prostaglandin E2 (Mello et al., 2002) and an inhibition of bradykinin induced plasma extravasation and swelling in the arthritic joint (Cruwys et al., 1994; Sharma et al., 1994). These findings along with the present study support the hypothesis that bradykinin

plays a key role in inflamed joint disease and may explain the beneficial effects of radiotherapy.

Since the results indicate that the applied dose has a high impact on treatment response and duration, the observations may be of clinical interest. Our *in vitro* study is supported by the clinical observation that low dose irradiation (0.5 Gy) induced immediate anti-inflammatory response in arthritis of the hand. Yet, a long lasting inhibition of chronic inflammatory disorders was only achieved with higher fractionated total doses of 2 Gy and more applied on the other hand (Seegenschmidt et al., 2000). This might suggest that a dose of 0.5 Gy is more suitable in the therapy of acute inflammatory benign diseases.

Nevertheless, since this study was initially planned as a pilot study including only limited amount of time points and doses, the results have to be interpreted with caution. A larger study design including more replicates and closer time points is clearly warranted.

In conclusion we were able to demonstrate a dose- and time-dependant change in bradykinin receptor expression on HF-15-cells in response to Co^{60}-irradiation. The results may imply radio-biological explanations for the beneficial effect of radiotherapy in inflammatory diseases like inflamed or degenerative joint disease.

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