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DIFFERENT INDUCTION OF DUAL CORTICOSTEROID RECEPTOR SYSTEM IN THE RAT HIPPOCAMPUS FOLLOWING GAMMA RADIATION

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Abstract

Cranial radiotherapy (CRT) is an effective way to prevent CNS relapse in children with acute lymphoblastic leukemia (ALL). However, CRT also has serious side effects on normal tissues, including long-term neuroendocrine disturbances. In order to test this clinical protocol on animals, we examined the effects of CRT (10 Gy) on the level of mRNA for glucocorticoid receptor (GR) and mineralocorticoid receptor (MR) in the hippocampus of 8-days-old rats. Irradiation rapidly stimulated GR gene expression in a time-dependent manner, whereas the time-course of MR mRNA expression showed no statistically significant changes. At postnatal day 42, the level of GR mRNA was diminished while the level of MR mRNA remained unchanged compared to matched controls. Dexamethasone suppression test (DST) revealed the altered nucleocytoplasmic shuttling of activated GR after CRT in 42-days-old rats, as a long-term consequence of gamma irradiation.

Introduction

Ionizing radiation (IR) has proven to be a powerful tool in the treatment of childhood cancer. Children with acute lymphoblastic leukemia receive CNS therapy to improve long-term survival. Neurotoxic effects, such as cognitive impairment, have been associated with this therapy [1]. Glucocorticoids (GCs) are frequently used as cotreatment agent because they may have potent proapoptotic properties and reduce nausea, hyperemesis and acute toxicity of normal tissue [2]. However, little is known about the induction of dual corticosteroid receptor system in the hippocampus after radiation exposure. Glucocorticoid receptor (GR) activation induces apoptosis of the granule cells in the hippocampus. Unlike GR activation, neuroprotection is seen after mineralocorticoid receptor (MR) activation [3]. To this aim, we examined the level of mRNA for GR and MR in the hippocampus of 8-days-old rats after irradiation of 10 Gy (^{60}Co γ-source) by semi quantitative RT-PCR using as the internal standard mRNA for glyceraldehyde-6-phosphate dehydrogenase (GAPDH). The hippocampus was isolated at the following post-irradiation times: 1 h, 2 h, 4 h, 8 h, 24 h and at postnatal day 42 in order to estimate long-term effects of γ-irradiation. The level of GR protein was detected by Western blot after treatment with synthetic glucocorticoid hormone (dexamethasone) in order to estimate a hypothalamic-pituitary-adrenal (HPA) axis response to radiation.

Results and Discussion

Therapeutic brain irradiation can cause progressive decline in cognitive function, along with impaired verbal and visual-spatial memory abilities, attention, organization and motor output [1]. Many of these functions, especially cognitive function and memory processes, are associated with action of corticosteroids (CS) in hippocampus. Two types of CS receptors are found in the brain: glucocorticoid receptor (GR) and mineralocorticoid receptor (MR). MR occupation is essential for the maintenance of basal activity in hippocampal circuit. In contrast to MR, GR becomes occupied during stress, thus promoting stress response. Differential activation of this dual receptor system may account for the opposing action of CS on neuronal proliferation, survival or death in hippocampal neurons [3]. The results presented herein clearly show that a clinically relevant dose of ionizing radiation [1] differently affects gene expression of the two corticosteroid receptors in the rat hippocampus. The prominent increase of GR mRNA after CRT (Fig. 1A) probably results in the activation of the GR-mediated apoptotic signaling pathway [3] which will be confirmed (or rejected) by our future analysis of the ratio between proapoptotic (Bax) and antiapoptotic molecules (Bcl-2) after CRT.

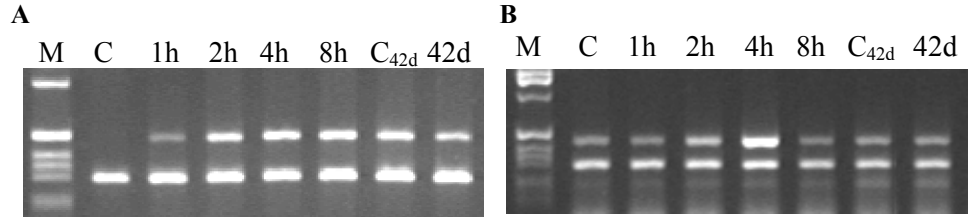

Fig. 1. Level of hippocampal GR mRNA (A) and MR mRNA (B) expression in rats irradiated with 10 Gy at different time points. The densitometric analysis of GR mRNA and MR mRNA were normalized to GAPDH (lower line).
M-DNA molecular weight marker
C-control (8-days-old rats)
C_{42d}- control (42-days-old rats)
42d-42-days-old rats, irradiated at the age of 8 days

Decrease of GR mRNA at postnatal day 42 (Fig. 1A, line C_{42d}, line 42d) points to a long-term attenuation of hypothalamic-pituitary-adrenal axis on the level of glucocorticoid negative feedback. Transient induction of MR mRNA, in contrast to induction of GR gene, revealed that the basal homeostasis response (mediated by MR) is not altered after CRT (Fig. 1B). This is confirmed by the unchanged level of MR mRNA in 42-days-old rats, irradiated at 8-days of age (Fig. 1B, line C_{42d}, line 42d).

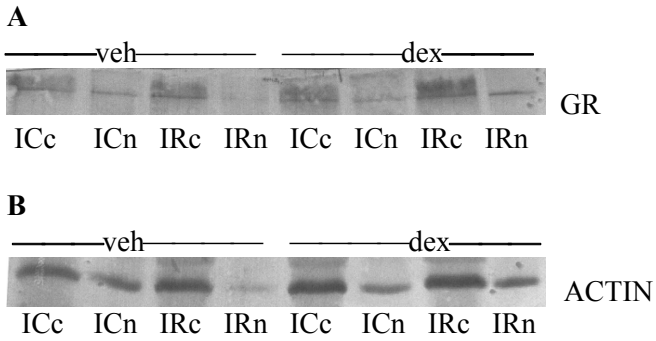

Fig. 2. Level of hippocampal GR (A) and actin (B) protein expression in 42-days-old rats irradiated with 10 Gy at age of 8 days. The densitometric analyses of GR protein were normalized to actin.
IC-immobilized control rats ; IR-irradiated rats
c-cytosolic proteins; n-nuclear extract proteins
veh-vehicle; dex-dexamethasone

These results were accompanied by altered nucleo-cytoplasmic shuttling of GR protein as revealed by Western blot (Fig. 2). The impaired translocation of activated GR (after treatment with dex) to nucleus in hippocampus of 42-days-old irradiated animals implicate to long-term attenuation of hypothalamic-pituitary-adrenal axis on the level of proteins.

Conclusion

According to these results, we may conclude that 10 Gy radiation treatment significantly affects the GR receptor system, both at the level of mRNA and protein, whereas the MR receptor system is not altered, thus probably leading to impairment of cognitive function and spatial learning. The attenuated HPA axis assessed late after brain irradiation may be involved in delayed brain response and could contribute to the observed clinical symptoms [1].

Acknowledgement

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