ORIGINAL ARTICLE

Effect of radiation on brain tissue endothelin-1 level and tumor development

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ABSTRACT

Background: Radiotherapy causes injury in the endothelial cells of blood vessels and the production of vasoactive amines such as endothelin-1 (ET-1). ET-1 is an important peptide in cancer development. In this study, the effects of radiation on brain tissue ET-1 level were evaluated. Is it possible to suggest a mechanism using ET-1 level in the production of this adverse effect? In this paper, the relationship between the development of brain tumors and the ET-1 level has been discussed.

Materials and Methods: Twenty-eight adult Sprague Dawley rats were used in the experiments. The rats were divided into four groups ($n = 7$) as follows: control group: radiation was not applied during the experiment; Group 1: Decapitated on the 1st day following radiation; Group 2: Decapitated on the 7th day following radiation; and Group 3: Decapitated on the 30th day following radiation. ET-1 levels were measured with enzyme-linked immunosorbent assay (ELISA) method. The *t*-test, variance analysis, and Tukey honestly significant difference (HSD) tests were used in the statistical analysis. A value of $P < 0.05$ was accepted as significant.

Results: No statistical differences were observed in the tissue ET-1 levels between the control group and other groups. According to the variance analysis and Tukey test, the differences between the groups were not significant.

Conclusion: We observed in this study that the effects of radiation on brain tumor development or malignant transformation are not mediated by ET-1 levels. In addition, these results support the hypothesis of the fact that medical treatment with ET-1 antagonists in clinical cases receiving radiotheraphy is unnecessary.

Key words: Brain tumor, endothelin-1, radiotheraphy

Introduction

Endothelial cells are the key cells of the vessel wall and are present in all levels of the vascular tree. They are essential for vascular functions. Radiotherapy causes injury to the contractile elements and endothelial cells of blood vessels. Production of vasoactive amines such as endothelin-1 (ET-1) is shown to increase in the human endothelial cells exposed to radiation in *in vitro* studies.[1] ET-1 has an important role in cancer biology with its effects on cell proliferation and apoptosis and its interactions with many cytokines.[2]

Radiotherapy is used successfully in the treatment of many primary and metastatic brain tumors. However, its adverse effects cannot be ignored. Exposure to radiation is known to be associated with carcinogenesis.[3,4] In addition to the development of primary tumors, the effect of radiation in transforming low-grade glial tumors to glial tumors with higher grades has also been discussed.[5] No studies in the literature have evaluated the effects of radiation on the brain tissue endothelins and discussed the association of this effect with tumor development.

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In this study, the early and late effects of radiation on brain tissue ET-1 level were evaluated and the relation of the development of brain tumors or malignant transformation in the glial tumor with ET-1 level has been discussed.

Materials and Methods

Experimental animals

Twenty-eight adult Sprague Dawley rats of age 4-6 weeks were used in the experiments. They were housed at a temperature of 22-25°C and in 12-h dark and 12-h light cycle. All phases of the experiment were performed in accordance with the legislation approved by the institutional review board.

Experimental groups

Rats were divided into four groups as control, Group 1, Group 2, and Group 3 rats.

Control group ($n = 7$)

Radiation was not applied during the experiment in this group. They were decapitated at the end of the experiment.

Group 1 ($n = 7$)

This group was decapitated on the 1st day following radiation and the brain tissues of the rats were excised.

Group 2 ($n = 7$)

This group was decapitated on the 7th day following radiation and the brain tissues of the rats were excised.

Group 3 ($n = 7$)

This group was decapitated on the 30th day following radiation and the brain tissues of the rats were excised.

Radiation application

A single dose of 7 Gy radiation was administered to the cranial region of the rats using an Electa-Precise Lineer accelerator device.[6]

Obtaining samples

After weighing, rats in all the groups were decapitated under ketamine (75 mg/kg) + xylazine (10 mg/kg) intraperitoneal (i.p.) anesthesia. Brain tissues of the rats were rapidly excised following decapitation. The extracted brain tissues were stored at −80°C for biochemical evaluation.

Biochemical evaluation

The obtained tissue samples were homogenized in phosphate-buffered saline (PBS) with anti-protease (5 μg/ml aprotinin) following washing with 0.9% NaCl and drying (1:9; w/v). After homogenization on ice, the samples were centrifuged (for 10 min at 4000 rpm and 4°C), and the ET-1 levels were measured in the supernatants obtained with ELISA method using rat ET-1 (Endothelin-1 EIA kit, Lot: 02011160A; ENZO, Lorrach, Germany) commercial kits. The tissue ET-1 levels obtained were evaluated as pg/g tissue.

Statistical analysis

Statistical Package for Social Sciences (SPSS) version 15.0 was used in the statistical analysis. The *t*-test, variance analysis, and Tukey honestly significant difference (HSD) test were used for comparisons between the all groups. A value of $P < 0.05$ was accepted as significant.

Results

This study was performed in 28 rats divided into four groups and the ET-1 levels obtained are shown in Table 1.

No statistical differences were found in the tissue ET-1(pg/g tissue) levels between control group and Group 1 [Table 2], control group and Group 2 [Table 3], control group and Group 3 [Table 4], Group 1 and Group 2 [Table 5], Group 1 and Group 3 [Table 6], and between Group 2 and

Table 1: Brain tissue endothelin-1 levels

	Tissue ET-1 levels (pg/g tissue)
Control	
1	212.3958
2	254.5194
3	894.5106
4	227.3363
5	498.3238
6	249.0569
7	309.2593
Group 1	
1	235.3527
2	275.2247
3	273.6863
4	203.9712
5	362.5609
6	243.14
7	168.2352
Group 2	
1	282.3308
2	263.4241
3	312.9596
4	271.4408
5	172.4282
6	202.5017
7	226.2494
Group 3	
1	194.6772
2	219.7616
3	296.6531
4	247.283
5	304.1107
6	189.9819
7	465.3098

Table 2: *t*-Test results showing comparison of the control group and Group 1

	Control (mean±SD)	Group 1 (mean±SD)	*P*
Tissue	377.915±247.650	251.738±61.795	0.215[ns]

[ns]*P*>0.05, SD – Standard deviation; ns – Not significant

Group 3 [Table 7]. According to the variance analysis and Tukey test, differences on days between the groups were not significant ($P > 0.05$) [Tables 8 and 9].

Discussion

ET-1 is an important peptide with biologic functions such as development, cellular proliferation, apoptosis, and cancer.[2]

Table 3: *t*-Test results showing comparison of the control group and Group 2

	Control (mean±SD)	Group 2 (mean±SD)	*P*
Tissue	377.915±247.650	247.334±49.052	0.196^{ns}

^{ns}P>0.05, SD – Standard deviation; ns – Not significant

Table 4: *t*-Test results showing comparison of the control group and Group 3

	Control (mean±SD)	Group 3 (mean±SD)	*P*
Tissue	377.915±247.650	273.968±95.765	0.321ns

^{ns}P>0.05, SD – Standard deviation; ns – Not significant

Table 5: *t*-Test results showing comparison between Group 1 and Group 2

	Group 1 (mean±SD)	Group 2 (mean±SD)	*P*
Tissue	251.738±61.795	247.334±49.052	0.885^{ns}

^{ns}P>0.05, SD – Standard deviation; ns – Not significant

Table 6: *t*-Test results showing comparison between Group 1 and Group 3

	Group 1 (mean±SD)	Group 3 (mean±SD)	*P*
Tissue	251.738±61.795	273.968±95.765	0.615^{ns}

^{ns}P>0.05, SD – Standard deviation; ns – Not significant

Table 7: *t*-Test results showing comparison between Group 2 and Group 3

	Group 2 (mean±SD)	Group 3 (mean±SD)	*P*
Tissue	247.334±49.052	273.968±95.765	0.525^{ns}

^{ns}P>0.05, SD – Standard deviation; ns – Not significant

Table 8: Variance analysis between the groups

	Sum of squares	Degree of freedom	Sum squares	F	*P*
Between the groups	78749.268	3	26249.756	1.368	0.276
In groups	460359.088	24	19181.629		
Overall	539108.356	27			

Table 9: Tukey test between the groups

	Control (mean±SD)	Group 1 (mean±SD)	Group 2 (mean±SD)	Group 3 (mean±SD)
Tissue	377.915± 247.65^{ns}	251.739± 61.795^{ns}	247.334± 49.052^{ns}	273.739± 95.765^{ns}

^{ns}P>0.05, SD – Standard deviation; ns – Not significant

It is a growth factor related to many tumor types including those of prostate, ovary, colon, cervix, breast, kidney, lung, and central nervous system and its plasma levels have been found to be increased in these tumors.[7] ET-1 stimulates DNA synthesis and cellular proliferation in several cells such as vascular smooth muscles, osteoblasts, glomerular mesengial cells, fibroblasts, and melanocytes, and also, it is a mitogen for different cell types including prostate and cervical cancer cells.[8]

The mitogenic effect of ET-1 might have a synergistic effect with other growth factors including epidermal growth factor (EGF), basic fibroblast growth factor (bFGF), insulin, insulin-like growth factors (IGFs), platelet-derived growth factors (PDGFs), transforming growth factors (TGFs), and interleukin-6 (IL-6).[9] ET-1 is accompanied by increases in pro-inflammatory cytokines such as tumor necrosis factor-α (TNF-α) and gamma interferon (IFN-γ).[9]

Various cytokines and vasoactive amines such as ET-1 are released from cells exposed to radiation. Radiation has been known to produce primarily brain tumors or cause malignant transformation of these tumors.[3-5] Is it possible to suggest a mechanism using ET-1 level in the production of this adverse effect? This report shows that there are no changes on the ET-1 levels after radiation. In addition, these results support the hypothesis of the fact that medical treatment with ET-1 antagonists in clinical cases receiving radiotherapy is unnecessary and ineffective.

Qi *et al*. reported that ET-1 levels increased in the blood of rats that received 15 Gy radiation, starting from day 5 and increasing to a maximum on day 60.[10] Merlin *et al*. reported increased ET-1 levels after exposing rats to radiation (1000 cGy and 2000 cGy) in the prostate and penile tissues, starting from day 20.[1] In this study, we used the 1st, 7th, and 30th day ET-1 level measurements. No increase was observed in the ET-1 levels of rats that received radiation compared to the control group; on the contrary, there was a decrease in ET-1 levels in the radiated group compared to the control group. Tsuboi *et al*. reported in their study that with high dose of radiation, ET-1 levels were suppressed due to cell damage.[11] Although the radiation dose used in this study was lower, a decrease in the ET-1 level was observed. The absence of a marked difference in the brain ET-1 levels in the early or late period after radiation might be due to the histologic characterization of the brain tissue. However, the dose of radiation that was applied might as well have a significant role. Therefore, the results of this study should be supported with further studies performed with different doses of radiation.

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Conflicts of interest

There are no conflicts of interest.

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