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Radiation-induced lowered neurogenesis associated with shortened latency of inhibitory avoidance memory response

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Abstract

The neural system is less sensitive to radiation than other late-responding organs and tissues such as the kidney and lung. The generation of new neurons in the adult mammalian brain has been documented in several works. Many studies show that adult hippocampal neurogenesis relates to hippocampal function, in several ways. In this study, we assessed the effect of single and fractionated cobalt radiation on neurogenesis in the dentate gyrus of the hippocampal formation.

The irradiation time for delivering 2 Gy (for fractionated dose radiation) and 10 Gy (for single dose radiation) at maximum depth were respectively 1.98 min and 9.92 min. To study the association with memory function we examined inhibitory avoidance memory using a step-through device. Brains were withdrawn and fixed, and then sections were stained with cresyl violet for neurons.

We found that a 10 Gy dose can induce lower neurogenesis in the dentate gyrus of the hippocampus ($p < 0.05$), in such a way that a fractionated dose (5 fractions of 2 Gy) is more effective than a single dose (one fraction of 10 Gy). Moreover, a fractionated dose could reduce step-through latency corresponding to damaged inhibitory avoidance memory ($p < 0.05$). Synergic action of an anaesthetic drug may be the cause of more reduction of neurogenesis in fractionated irradiated rats. There was no significant difference in latency of the inhibitory avoidance memory response between the single 10 Gy group and the sham group, while fractionated 10 Gy could reduce latency. Different mechanisms of action in the two regimens of irradiation may be a reason.

Key words: *cobalt radiation, neurogenesis, step-through, dentate gyrus, inhibitory avoidance memory.*

Introduction

Radiation therapy is one of the first choices of treatment for intracranial tumours, which is restricted by damage to normal brain tissue [7,12]. Of course, the mechanism of this damage remains unclear; however, the number of studies on this issue is increasing [26]. Certain glial, neural, and endothelial cells in the CNS after irradiation suffer from apoptosis [4,10-12,18,29]. Radiation-induced lowered neurogenesis of hippocam-

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pus in rodents has been evidenced by many authors [1,14,15].

A number of studies have shown increased hippocampal neurogenesis in response to its function [3,6,22]. Trained rats have a higher rate of neurogenesis [28]. Dentate granular neurons derive from precursor cells located in the subgranular zone [5]. Knowing radiation effects on neurogenic cells may provide a tool to understand specific consequences associated with cranial radiation.

Months to years following radiotherapy many patients may suffer from damaged short-term memory, spatial relations, visual motor processing, quantitative skills and attention [23]. Damage to neural precursor cells may be responsible for some of the above consequences [14,15,20].

In the mammalian brain two regions have active neurogenesis: the subventricular zone of the anterior lateral ventricles and the subgranular zone of the dentate gyrus in the hippocampus [5,25].

Neurogenic cells in dentate subgranular cells are sensitive to ionizing radiation, undergoing apoptosis after doses ranging from 0.4 Gy to 18 Gy [13,16,21,23, 24,27].

Madsen *et al*. reported that subjecting adult rats to fractionated brain radiation blocked the formation of neurogenesis in the dentate gyrus of the hippocampal formation [10, 14]. Animals with blocked neurogenesis showed poorer performance in a hippocampus-dependent place recognition task. Other reports documented the functional significance of newly generated neurons in the dentate gyrus.

In this study, we assessed the effect of single dose radiation (10 Gy) and fractionated dose radiation (five fractions of 2 Gy) on neurogenesis in the dentate gyrus of hippocampal formation.

Material and methods

Animals

Forty-two adult male Wistar rats, weighing 200-250 g, were used in the study. They were given free access to normal laboratory chow and water. Temperature of the animal house was 22 ± 3°C. The rats were kept in groups of six.

Irradiation

The radiation machine was a cobalt 60 unit routinely used for radiotherapy. Its output was 115.28 cGy/min at SSD of 80 cm at maximum depth. The field size was 5 × 5 cm^2 including the shielded area. The irradiation time for delivering 2 Gy (for fractionated dose radiation) and 10 Gy (for single dose radiation) at maximum depth was respectively 1.98 min and 9.92 min.

Eyes of rats were shielded by lead blocks to avoid any ophthalmic reaction interrupting the memory test.

Inhibitory avoidance memory apparatus

The apparatus consisted of two chambers of the same size (20 × 20 × 30 cm) separated by a dividing wall, a guillotine door (7.9 cm^2) which could be lifted manually. The walls and floor of one chamber were made of white opaque resin, while the other was dark. The floor of the dark chamber was made of stainless steel rods (3 mm in diameter and 1 cm intervals). Intermittent electric shocks (50 Hz, 3 s, 1 mA intensity) were delivered to the grid floor of the dark chamber by an isolated stimulator.

Behavioural test

All animals were allowed to habituate in the experimental room for at least 30 min prior to the experiments. Then each rat was lightly placed in the bright chamber. After 5 s the guillotine door was opened and the animal was permitted to enter the dark chamber. The latency with which the animal entered the dark chamber was recorded. Animals that delayed more than 100 s to enter the dark chamber were excluded. When the rat crossed with the four paws to the dark chamber, the guillotine door was closed and the rat was quickly removed from the dark chamber.

After 30 min when the rat crossed to the dark chamber, the guillotine door was closed and a foot electric shock was delivered to the grid floor of the dark room. After 20 s the rat was removed from the apparatus and placed temporarily in the home cage.

After 2 min the animal was tested again by the same method as before. If the rat did not enter the dark chamber within 120 s a successful acquisition of the response was recorded. Otherwise when the rat entered the dark chamber with a delay less than 120 s the door was closed and the second shock was delivered. If the rat passed the second test successfully, it would be accepted for the experiment. Rats with delays less than 120 s were rejected.

To examine inhibitory memory each rat was placed in the bright chamber, the guillotine door was opened after 5 s and latency of entering the dark room was taken as the evaluation score. The longest latency to enter the dark room (place of receiving the shock) was considered as full memory, which normally is 300 s.

Histology

Tissue processing: After behavioural testing rats were decapitated under diethyl ether anaesthesia. Brains were withdrawn and then fixed for two weeks in 10% formaldehyde. Different degrees of alcohol were used for dehydration followed by clarification with xylol. After histological processing, tissue was impregnated and then embedded in paraffin wax.

The 7 μm coronal sections were serially collected from Bregma –3.30 mm to –6.04 mm of the hippocampal formation. An interval of 20 μm was placed between each two consecutive sections.

The sections were stained with cresyl violet in accordance with routine laboratory procedures [2].

A photograph of each section was produced using an Olympus BX 51 microscope and a DP 12 digital camera under a magnification of 1000. An area of 3600 μm^2 was selected in the lower horn of the dentate gyrus in all sections. To measure the area density of the granule cells, the images were transferred to the computer. Using OLYSIA Autobioreport software, Olympus Co, the appropriate grids were superimposed on the pictures and the cells were counted manually. To perform an unbiased measurement, the individual was double-blinded and only the cells with significant granule cell characteristics were counted [8,9].

Statistical analysis

All the data were entered into and analysed by SPSS 11.5 software. The data were expressed as mean ± SEM. The statistical analysis was performed using one and two way analysis of variance (ANOVA). Post-hoc comparison of means was carried out with the Tukey test for multiple comparisons, when appropriate.

The level of statistical significance was set at $P < 0.05$. Calculations were performed using the SPSS statistical package.

Results

Avoidance inhibitory memory test

In experimental groups the rats were trained and one month later at the same time were tested. Table I shows the results of the memory test.

As the table shows, latency of response in the sham group given anaesthesia on 5 consecutive days without radiation was 258.66 ± 53.95 s while for the 2 × 5 Gy group and 10 Gy group it was respectively 43.83 ± 27.33 s and 203 ± 84.66 s, showing a significant difference between sham and fractionated (2 × 5 Gy) groups ($p < 0.05$). We found no significant difference between sham and single fraction (10 Gy) groups. Moreover, there was no significant difference between control and sham groups. Fig. 1 shows the results as statistical bars.

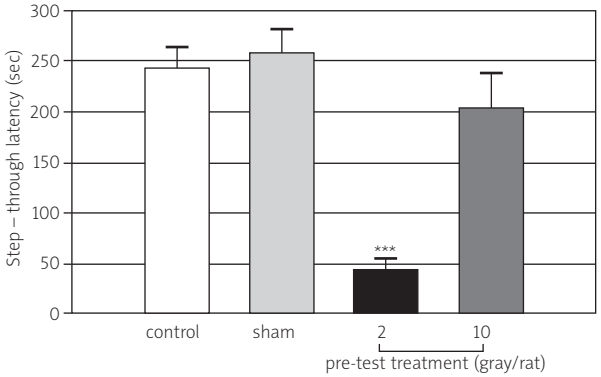

Fig. 1. Significance difference between sham and fractionated radiation.

Table I. Latency of entrance (in seconds) to dark chamber in different groups of rats

	Rat 1	Rat 2	Rat 3	Rat 4	Rat 5	Rat 6
Control	300.00	200.00	248.00	190.00	300.00	225.00
Sham	153.00	267.00	281.00	292.00	259.00	300.00
2 × 5 Gy	27.00	37.00	96.00	45.00	40.00	18.00
10 Gy	262.00	118.00	276.00	127.00	135.00	300.00

Table II. Mean number of neurons in all groups

	DG	Mean	Std. deviation	Area µm²
1	Control	22.98	3.278	3600
2	Sham anaesthesia	14.33	14.33	3600
3	Sham avoidance	19.58	2.571	3600
4	Rad 10 – avoidance	20.70	2.623	3600
5	Rad 2 × 5 – avoidance	16.45	1.934	3600
6	Rad 10 + avoidance	14.60	1.932	3600
7	Rad 2 × 5 + avoidance	12.22	1.874	3600

Cell counts (neurogenesis)

Table II shows the results of cell counts in different groups. Granular cells in the lower horn in the dentate gyrus were counted. The number of neurons in all study groups was lower than in the control group and the differences were significant ($P < 0.05$).

The analysis shows a significant difference between the sham anaesthesia group and the fractionated radiation group ($p = 0.002$). We found no significant difference between the single fraction radiation and sham anaesthesia group ($p = 0.999$), which is consistent with the memory test. Also we found that not only the avoidance test can reduce the neuron density, but also the radiation can do it (Table II).

Discussion

This study was performed to assess whether radiation can decrease the number of granular neurons proliferating in the dentate gyrus of the hippocampal formation, and if this can affect its function as inhibitory memory latency.

We observed good consistency between neurogenesis reduction and shortness of inhibitory memory latency. Five fractions of 2 Gy was more effective than single 10 Gy to reduce neurogenesis, while the same result was obtained during the inhibitory memory test. Our results showed that fractionated radiation is more effective than single radiation to shorten latency of the memory response.

Based on our results there was no significance difference between the number of granular neurons in the hippocampal formation after a single fraction of 10 Gy and the anaesthesia sham group ($P = 0.999$), a result which verified inhibitory memory test data.

Our results resemble those of another study that showed a decrease of cell density after the avoidance memory test [17]. It seems that three factors play roles in reduction of neurons in the dentate gyrus: anaesthesia, avoidance test and radiation.

Meanwhile there is a significant difference between the fractionated group and the anaesthesia group, suggesting that the anaesthetic drug behaves as an interfering parameter or additive agent to radiation. For early responding tissues (with high α/β ratio) there is little sensitization to fractionation [7], as we also observed. But more effectiveness of the fractionated regimen may be related to five administrations of the anaesthetic drug. Synergic action of the drug and radiation to kill neural cells in the hippocampal formation is a hypothesis which should be assessed and proved.

Madsen *et al*. observed blocked neurogenesis after 3 Gy irradiation. Animals with blocked neurogenesis performed poorer than controls in hippocampus-dependent function [14]. Our data showed the same effect on inhibitory avoidance memory, another hippocampus-dependent function.

Many researchers have addressed the potential effectiveness of neurogenesis as a cause of hippocampal functions of learning and memory [5,22,28]. According to our results there is a relationship between radiation-induced lowered neurogenesis and shortness of memory latency, giving a clue for management of radiotherapy consequences.

This was consistent with the results of Santarelli *et al.* and Raber *et al.*, who reported an association between decreased neurogenesis and cognitive deterioration [7,19,21].

Conclusions

We summarize our conclusions in five main topics:

1. There is good consistency between reduction of neurogenesis and shortness of the inhibitory memory response as a hippocampus-dependent function.
2. As we are dealing with an early responding tissue it is reasonable that there is low sensitization to fractionation.
3. Greater efficacy of the fractionated regimen to reduce neurogenesis may be due to synergic action of the anaesthetic drug.
4. No effect of single 10 Gy on reduction of latency of the inhibitory avoidance response, in comparison with a fractionated dose, may be due to different mechanisms of action in the two regimens.
5. Three factors play roles in reduction of neurons in the dentate gyrus: anaesthesia, avoidance test and radiation.

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