Protection against Radiation-Induced Performance Decrement in Mice

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ABSTRACT

Recognising that there is lack of information on the effects of low-level ionizing radiations and the modifying role of radioprotectors, an attempt has been made in this study to explore the relationship between impairment of spatial learning and low level of radiation exposure. A radial arm maze was utilised to evaluate radiation-induced behavioural alterations and performance decrement in mice. Immediately after whole body exposure to gamma radiation (absorbed dose, 1 Gy) significant perturbations in the learned behaviour of the animals were observed. The regular control movement became irregular and the food consumption time was reduced appreciably (40 %). Recovery took place in four days. If diltiazem (7 mg/kg b.w.), a Ca^{2+} channel blocker and a radioprotector, was administered i.p. 20-30 min prior to irradiation, radiation-induced behavioural abnormalities were reduced. Mechanisms underlying protection by diltiazem against radiation-induced performance decrement observed in the present study need to be investigated.

1. INTRODUCTION

Nervous system has been classically recognised as radioresistant. However, behavioural studies have shown decrement in performance and disorientation at doses of 5-10 Gy[1-3]. There is increasing evidence that ionizing radiation may alter neural physiology and function at doses substantially below those required to produce neurological changes and death. In experimental animals sufficiently low level (0.001 Gy) of radiation exposure was observed to cause transient loss of memory for a learned behaviour[4]. Reliable data on the effects of sublethal doses of radiation on human behaviour and performance decrements are not available.

Recently, it has been observed that hippocampal formation in medial temporal lobe of brain is essential for memory and learning processes[5]. Damage caused to mature hippocampus in patients due to surgery or cardio-pulmonary ischemia resulted in cognitive deficits characterised by severe amnesia and loss of learning[6-10] In rats, inflicted damage to hippocampus produces a particularly striking impairment in learning and remembering spatial information[11,12].

Prenatal exposure of developing brain to ionizing radiation has been shown to cause severe mental retardation and deficiency in work performance in humans[13-15]. It is not known whether ionizing radiation at sublethal doses could modify the functional status of adult mature hippocampus, resulting in transient loss of memory and deficits in performance. The present study has been designed to explore the relationship between impairment of spatial learning and low level of radiation exposure in adult mice and to develop

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strategies to prevent radiation-induced performance decrement.

2. MATERIALS & METHODS

2.1 Animals

Strain A, female mice, weighing 20 g, kept at 25 ± 2 °C ambient temperature, 12 hr photoperiod, pellet food (Amrut Laboratory Animal Feed, India) and water given *ad libitum*, were used. Ten animals each were used in control and experimental groups.

2.2 Equipment

For assessing spatial memory, a radial arm maze was used. The maze was fabricated at the Institute of Nuclear Medicine & Allied Sciences (INMAS), Delhi, from transparent perspex sheet, as described by SienKiewicz, *et al*[16]. with some modifications. The maze consists of eight identical arms radiating from a central platform. At the distal end of each arm, a small food well was provided for keeping food pellet. The maze was placed within a laboratory equipped with overhead fluorescent light, wall-mounted air-conditioner and containing assorted items, representing extra-maze cues. During trials, the animals were under constant remote surveillance.

2.3 Drugs

Diltiazem, a benzothiazepine used as calcium channel blocker in cardiovascular therapy, has been reported to give protection to mice against lethal gamma irradiation[17]. Diltiazem has been evaluated in the present study for its behavioural radioprotecting action.

2.4 Experimental Protocol

The mice were maintained on restricted food for 15 days till their initial body weight was reduced by 20 per cent. Water was made available *ad libitum*. During the last seven days of weight reduction, the animals were acclimatized to the maze. Animals explored the maze for 10 min each day and were allowed to consume the food reward (20 mg pellet) placed in each food well. Before the beginning of each daily training session, a pellet of food was placed in each food well. A mouse was placed in the central arena and allowed to move freely and consume the food in all the eight arms. After 10 to 15 sessions, the mouse learned to move in a regular fashion (clockwise or anticlockwise) and consumed the total food in a specific time (~ 5 min). After completion of the training, the mouse was exposed to 1 Gy whole body gamma irradiation from a cobalt-60 gamma source (Atomic Energy Commission, Canada). The dose rate during the experiment was between 2.0 and 1.7 Gy/s Control animals were sham-irradiated by putting them inside radiation chamber momentarily, shielded with 90 per cent attenuator. In the process, the total dose delivered was reduced to 0.003 Gy. All the animals were administered 0.2 ml normal saline i.p. (controls) or 0.2 ml normal saline containing diltiazem (7 mg/kg body weight), 20-30 min prior to irradiation. Immediately (60 s) after exposure to 1 Gy whole body gamma irradiation, the mice were placed in the central arena of the radial arm maze. Movement pattern and time taken for consumption of food in eight arms were observed.

3. RESULTS & DISCUSSION

Post-irradiation movement of the animals became irregular and haphazard (Fig. 1), though the animals consumed the total food in 3 ± 0.2 min rather than 5 ± 0.3 min in control animals (40 per cent reduction in time). The alterations in behaviour and performance gradually normalised over a period of four days (Figs 1 and 2). In another study, diltiazem, at a very low dose, was found to mitigate radiation-induced behavioural alterations in rats in terms of conditioned taste aversion for saccharin solution[18]. In the present series of experiments, diltiazem (7 mg/kg b.w.) was administered to mice 20-30 min prior to irradiation.

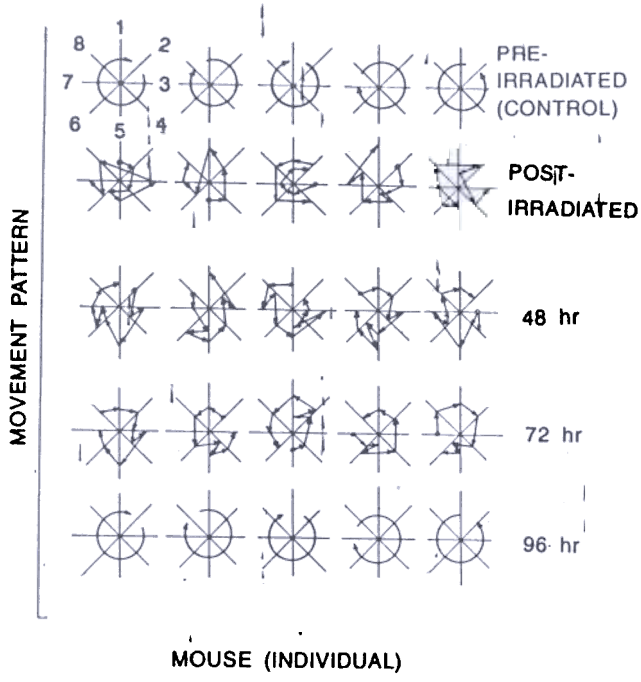

Figure 1. Schematic view of movement pattern of individual mouse 60 s after whole body exposure to 1 Gy gamma radiation and gradual recovery of radiation-induced alterations as a function of time. Controls were Sham-irradiated.

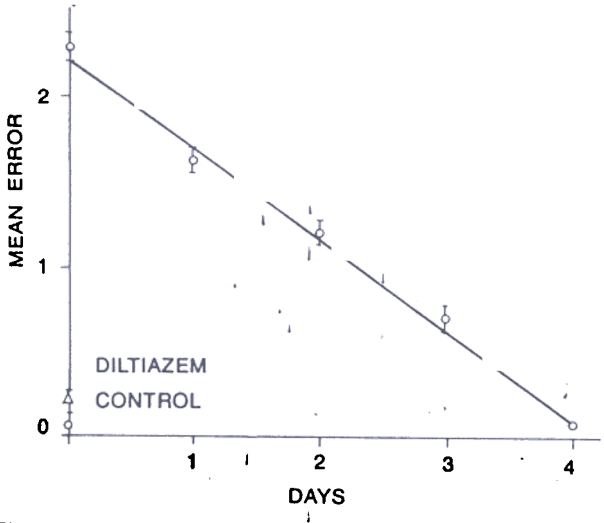

Figure 2. Radiation-induced behavioural changes and protective effects of diltiazem 7 mg/kg body weight administered i.p. 20-30 min before wholebody irradiation with gamma rays (1 Gy). Mean error is expressed in terms of revisits to the arms.

The results showed that diltiazem-administered mice reduced the frequency of errors in comparison to control animals (Fig. 2). It is of interest that at this dose level, diltiazem is not radioprotective against lethal gamma irradiation. The mechanisms underlying the loss of memory and learning immediately following irradiation (1 Gy) in the animals are not clear. It is known that hippocampus is involved in learning and memory processes. In *in vitro* experiments with rat hippocampal slices, extremely low levels of irradiation (< 0.008 Gy) have been observed to evoke spike discharges[19] Hippocampus has been long recognised as being exceptionally vulnerable to numerous insults[20] and various mechanisms, such as sparse microvasculature or aspects of highly sensitive hippocampal electrophysiology have been suggested as causative factors. Further work with hippocampal slices from guinea pigs have revealed that hippocampal neuronal physiology could be significantly altered by free radicals[21]. It is known that ionizing radiation generate highly reactive oxygen-free radicals, resulting in lipid peroxidation of cell membrane, impaired synaptic efficacy and spike generation, calcium influx into the cell, and cellular dysfunction. Lipid peroxidation was inversely related to dose rate[22] and low level of free radicals inhibits maintenance of long-term potentiation (LTP)[23] related to learning and memory processes. Taken together, it seems probable that diltiazem at a lower dose protects neuronal cell physiology by inhibiting calcium influx into the cell (leading to cascade of cellular dysfunctions), thereby improving radiogenic alteration in behaviour and performance.

The results of the present study suggest that a sublethal radiation dose could possibly alter normal functioning of hippocampus and thus influence learning behaviour of mice. At present, it is difficult to explain the effects of diltiazem on protection against radiation-induced performance decrement. However, the present observations have significant implications for radiation therapy, nuclear industry and rescue operations in cases of nuclear accidents. During diagnostic radiology or

treatment of brain primary tumours and metastasis, normal brain tissues are exposed to radiation. With the advent of better behavioural radioprotectants, quality of life in cancer patients might get improved. In rescue operations, personnel could be protected from radiation-induced deficits in behaviour and performance through prior administration of a behavioural radioprotectant.

More work is needed in this direction and is of paramount importance for the Defence Services.

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