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ORIGINAL ARTICLE

Alteration in Testicular acid phosphatase on administration of γ-radiation Swiss albino mice

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ABSTRACT

Activity of acid phosphatase in the present study was observed to change after exposure to various doses of γ- radiation. The value in control and radiated mice were after 0.05Gy, 0.10Gy, 0.15Gy, 0.20 Gy and 0.25Gy were 6.10 KA/unit, 8.55 KA/unit, 4.73 KA/unit, 10.83 KA/unit, 6.09 KA/unit and 4.67 KA/unite, respectively. Statistically, the significant value was 10.83 KA/unit. Increment in AcPase concentration may be suggestive of increased lysosomal activity, leaching of enzymes from lysed cells or necrotic changes due to phagocytic action of lysosomal enzymes. On the other hand, increased utilization of AcPase in cell organelles/tissues degradation of damage may account for attenuated AcPase functions may severely impair spermatogenesis.

Key words: *ionized radiation, lysosomal enzyme, testes*

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INTRODUCTION

Ionized radiation was the first to be recognized as environmental teratogen to effect the humans. Gamma radiations are one of the most penetrable emanations for biological tissues. Mammalian testes are an ideal organ to study a variety of cellular processes example cell division, growth, differentiation and maturation. Radiation induced damage to testes have been subject of absorbing interest in addition of emanation of natural radiations from earth crust, the increased use of radionucleotides in medicine, veterinary research, and therapies has increased the vulnerability and sensitivity of human, animals and plant population to radiation hazards [1]. This threat is real, since all cell and organisms have the inherent ability to bioamplification example mutation, cancer, tereta formation and cytogenetic aberrations [2 and 3]. They are known to cause a variety of oscillations in the cytoarchitecture, permeability, irritability, conductivity and metabolic status of cells. The extent of such perturbation in many cases appears to be dependent on dose related relationship. However, this is not always true due to heterogenicity of tissues and their physiological and biochemical status. Several physical, chemical and biological factors play a deterministic role in the radio sensitivity response. Several cytopathologies and aberrations in the enzyme-isoenzyme, and enzyme-substrate profile have been described in different somatic tissue type. The information on these aspects in gonodal tissues (exocrine and endocrine) is somewhat fractured, debatable and, therefore, needs further study in a variety of mammalian and other forms to arrive at some common and meaningful conclusion.

MATERIALS AND METHODS

Procedure of Radiation: Swiss albino mice were restrained in position by tying rubber bands around the fore, and hind limbs. They were exposed to single pulse of various doses of γ-radiation for different times by Cobalt-60 camera. Radiations were applied to the abdominal region at a point where the paired abdominal testes were located. Control groups were sham irradiated and maintained for comparison with γ-irradiated males under similar conditions.

Surgical Processes and Preparation of Testicular Homogenates: Mice of control and experimental groups were weighed before and after radiation. They were sacrificed by cervical dislocation after 24 h of radiation. Testes were surgically excised under aseptic conditions. They were freed off of excess fascia and blood clots, rinsed several times in chilled physiological saline (4°C). After blotting the tissue the wet weight of each test was separately recorded on monopan electric balance. Homogenate of testes (100 mg/ml) were prepared in normal saline (0.9 % w/v) in ice bath in Potter: Elvehjem homogenizer (for 5 min). The homogenate were centrifuge at 3000 rpm for 20 min to obtain the subcellular fractions. The supernatant was decanted and utilized for biochemical assay of acid phosphatase (Acpase) as per procedures detailed below.

Acid phosphatase (AcPase): Acpase in the testicular homogenate was estimated by Kings and Jagathesan's method [4].

Principle: AcPase from testicular homogenate converts phenyl phosphate to inorganic phosphate and phenol at pH of 5.0. The phenol so formed reacts in the acidic medium with amino antipyrine in the presence of oxidizing agent potassium ferrcynide and forms an orange red-coloured complex which is measured colorimetrically. The colour intensity is proportional to enzyme activity.

(i) Disodium phenyl phosphatase $\xrightarrow{AC\,pase}$ Phenol + Pi

pH 5.0 (Disodium hydrogen phosphate)

(ii) Phenol + 4-AA $\xrightarrow[OH^{-1}]{KCN}$ Orange-red coloured complex.

Procedure: Four test tubes labelled as 'Blank' (B), 'Standard' (S), 'Control' (C) and 'Test' (T) were set up in pairs. Buffered substrate pH 5.0 (0.5 ml) was added to 'C' and 'T'. Distilled water was added to 'B' (1.0 ml), 'S' (0.5 ml), 'C' (0.5 ml), and 'T' (0.5 ml). They were vortexed and incubated at 37°C for 30 min. Working phenol standard 10 mg % (0.5 ml) and tissue homogenate (1 ml) were added to the both tubes 'T' and 'S', respectively. They were vortexed and further incubate at 37°C for 60 min. Then 0.5 NaOH (0.5 ml) was added to all the tubes. At this stage tissue homogenate (0.1 ml) was added to 'C' and then 0.5 M sodium bicarbonate (0.5 ml) and 2.4% potassium ferricyanide (0.5 ml) were added to all tubes. The assay reagents were vortexed and O.D. was read at 570 nm.

Calculation:

$$\text{ACPase (in KA)} = \frac{\text{O. D. of Test} - \text{O. D. of control}}{\text{O. D. of Standard} - \text{OD of Blank}} \times 5$$

RESULTS

AcPase amounts in the testes of the control and experimental groups displayed dose related alterations in their minimal and maximum values in response to challenge by 0.05 Gy and 0.1 Gy of γ-radiation. These values were computed to be 8.55 K.A./Unit and 10.83 K.A./unit, respectively (Table 1). However, exposure to 0.1 Gy, 0.2 Gy and 0.25 Gy caused a decrease in AcPase amounts and they were estimated to be 4.73 K.A./unit, 6.09

K.A./unit and 4.67 K.A./unit, respectively vis-a-vis control which was 6.10 K.A./unit (Table 1).

DISCUSSION

The quantitative estimates of total testicular AcPase in the testes of male Swiss albino mice challenged by various dose of y-radiation were observed to increase in response to challenge by 0.05 Gy and 0.15 Gy. In percentage terms, this increment was 24.13% and 21.81%, respectively. However, AcPase values on exposure to 0.1 Gy, 0.2 Gy and 0.25 Gy were computed to be 43.58%, 23.60% and 28.25%. As compare to control (100%) they manifested a cremental trend.

Elevation of AcPase concentration may be linked with increased lysosomal activity, leaching due to necrotic change or due to phagocytic action on the fragile and deranged cellular organelles affected by radiation [5]. Lysosomes are implicated in intracellular protein digestion and can also cause autophagic digestion of cellular organelles or the cell itself *i.e.,* autolysis [6 and 7].

Table 1: Quantitative shifts in the amount of acid phosphatase (KA)in the testis of Swiss albino mice exposed to various doses of ^{60}CO-γ-rays

S. No.	Dose (Gy)	AcPase(KA)
1.	Control	6.103±0.272
2.	0.05	8.553±0.388
3.	0.10	4.737±0.135
4.	0.15	10.830±0.217
5.	0.20	6.090±0.365
6.	0.25	4.673±0.340

Values are mean ± S.E., KA = Kinetic activity

Radiation induced cellular degradation of tissue damage was shown by Hugan and Borger, Wills and Wilkinson and Dhawan et al. [8, 9 and 10]. This was shown to cause increase in acid phosphatase activity. An increased activity of acid phosphatase after irradiation has also been reported by Shah and Gadhia [11]. The increment in the activity of AcPase in mammalian tissue was reported by Samarth and Samarth [5] Kroll and Nipper [12], Samarth et al., [13] due to damage to lysosomes which are believed to be responsible for cell death, and this is released by necrotic cells. These reported results are in close agreement with the present investigations. In addition, Nehru et al. [14] reported a significant decrease in the activity of AcPase at 2.5Gy and 10Gy doses. This decrement in the activity was correlated with the state of germ cell population in the seminiferous tubule which was found to be depleted with time. This observation is compatible with the results obtained in the present studies.

The pattern of AcPase fluctuations appear to be related well with the structural profile of testes, process of spermatogenesis and androgenesis. AcPase is a 'marker' enzyme for lysosomes. Its patterns and shifts in them can be linked to injury and necrosis of testicular cells in various stages of growth, division, spermiogenesis', maturation and release.

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