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ORIGINAL ARTICLE

Effect of gamma radiation on alkaline phosphatase enzyme in Swiss albino mice

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ABSTRACT

Alkaline phosphatase (Alkpase) is an androgen-dependent enzyme. Experimental groups its value was observed to be variable consequent to γ-radiation. In control mice and ones radiated by 0.05 Gy, 0.10 Gy, 0.15 Gy, 0.20 Gy and 0.25 Gy the values were computed to be 22.83 KA/unit, 28.34 KA/unit, 27.81 KA/unit, 12.88 KA/unit, 17.34 KA/unit, 16.28 KA/unit respectively. Statically the significant change was induced by 0.25 Gy and it was 16.28 KA/unit. At other dose level there were oscillations in Alkpase values. This may possibly be due to differential sensitivity of cell and tissue type of testes. However, increase of Alkpase at some doses indicates increased phosphorylation, membrane permeability and transfer of metabolites in the testicular cells. These may alter androgenic functions of the leydig cells. However at higher dose significant decrease in Alkpase amounts may mean attenuated 'turnover' of androgen. This would have serious effects on libido and sexual behavior.

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

INTRODUCTION

Ionized radiation was the first to be recognized as environmental pollutant to effect the living organisms. Gamma radiations are one of the most penetrable ionized radiations for biological tissues. The consequential effect of this is characterized by mutation and cell cycle delay. Loss of reproductive abilities and even survival are the long term effects of these cellular and molecular pathologies (Hittleman *et al.* 1980, Fowler 1989 and El-Benhawy *et al.* 2015). 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 (Wilmink and Grundt 2011). This threat is real, since all cell and organisms have the inherent ability to bioamplification e.g mutation, cancer, tereta formation and cytogenetic aberrations (Eberhard et al. 2013 and Comish et al. 2014). 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: The animals 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 testes 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 alkaline phosphatase (Alkpase) as per procedures detailed below.

Alkaline Phosphatase (AlkPase): AlkPase was estimated by Kind and King's method (Kind and King 1954).

Principle: AlkPase in the testicular homogenate converts phenyl phosphate to inorganic phosphate and phenol at pH 10.0. The phenol so formed reacts in alkaline medium with 4-amino antipyrine in the presence of the oxidizing agent potassium ferricyanide and forms an orange red coloured complex which can be measured colorimetrically. The colour intensity is proportional to the enzyme activity.

$$\text{Phenyl phosphate} \xrightarrow[\text{pH 10.0}]{\textit{Alk.Pase}} \text{Phenol + Pi (Disodium hydrogen phosphate)}$$

$$\text{Phenol + 4-aminoantipyrine} \xrightarrow[OH^{-1}]{KCN} \text{Orange red coloured complex}$$

Procedure: Four test tubes labelled as 'Blank' (B) 'Standard' (S), 'Control (C) and 'Test'(T) were set up in pairs. Buffered substrate pH 10.0 (0.5 ml) was added to 'C' and 'T'. Distilled water was added to 'B' (1.00 ml), 'S' (0.55 ml) 'C' and T' (0.5 ml), they were vortexed and incubated for 3 min. at 37 °C. Phenol standard 10 mg% (0.5 ml) and tissue homogenate (0.05 ml) was added to 'S' and 'T' respectively and vortexed; and further incubated for 15 min at 37 °C. Then the chromogen reagent (1.0 ml) was added to each tube. Tissue homogenate (0.05 ml) was added to control 'C'. The assay reagents were vortexed and O.D. was recorded at 510 nm.

Calculation

$$\text{Testicular AlkPase(K.A. units)} = \frac{\text{O.D. of Test} - \text{O.D. of Control}}{\text{O.D. of Standard} - \text{O.D. of Blank}} X\ 10$$

RESULTS

The biochemical profile of total testicular alkaline phosphatase in response to challenge by various doses of γ-radiation manifested characteristics patterns. For the sake of clarity each of these is narrated as under:

Alkaline phosphatase (AlkPase): Alkpase values were observed to display fluctuating minimal and maximal order. The concentration of testicular AlkPase in control was 22.83 K.A./unit (Table 1). The concentration of testicular AlkPase was significantly increased when challenged by 0.05 Gy and 0.1 Gy of irradiation. It was 28.34 K.A./unit and 27.81 K.A./unit, respectively (Table 1). In group IV, V and VI irradiated by 0.15 Gy, 0.2 Gy and 0.25 Gy the values of AlkPase were computed to be 12.88 K.A./unit 17.34 K.A./unit, 16.28 K.A./unit (Table 1).

DISCUSSION

Total testicular Alkpase of Swiss albino mice in various experimental groups were observed to be variable. Thus, in the y-irradiated mice a significant rise in the Alkpase amounts was computed after exposure to 0.05 Gy and 0.1 Gy to be 24.13% and 21.8% respectively vis-a-vis control. In groups IV, V and VI irradiated by 0.15 Gy, 0.2 Gy. 0.25 Gy, the values were computed to be decreased by 48.58%, 23.60% and 28.25% as compare to control.

Table 1: Quantitative alterations in the amounts of testicular Alkpase (KA unit) in Swiss albino mice challenged by various doses of ^{60}CO-γ-rays

S. No.	Dose (Gy)	Alkpase (KA units)
1.	Control	22.837±0.408
2.	0.05	28.343±0.596
3.	0.10	27.817±0.726
4.	0.15	12.817±0.457
5.	0.20	17.343±0.337
6.	0.25	16.283±0.222

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

Alkpase is lysosomal enzyme which is believed to be androgen dependent and is often linked to "tides" and "ebb" in the concentration of these hormones. Its increase has been considered as an indicator of cellular injury (necrosis). Since enzyme play an important role in dissolution of dead cells of body (Kaplan 1972), elevation in the activity may be linked with cell death, derangement and dysfunction of germ cells and Leydig cells.

Radiation-induced stress is also considered to be responsible for increase in the activity of this enzyme. A comparison of the present finding with other published information indicates several parallels as well as differences.

Thus, a significant increase in Alkpase activity/gm testes at 2.5 and 10 Gy does was observed by Nehru et al. (1991), but this increment showed recovery after 16 weeks post irradiation.

The fluctuation in the pattern of Alkpase activity also depends on the structural profile of testis. Radiosensitivity of different cells of spermatogenesis was observed to be different i.e., spermatogonia are highly radiosensitive. This radiosensitivity decreased with the maturation of cells. Spermatidare known to be relatively radioresistant (Lataillade *et al.* 1991 and Kangasniemi *et al* 1996). However, if cells are in premature stage extent of the damage would be large.

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