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Gamma Radiation Induced Alteration in Biochemical Milieu of Testes of Sexually Matured Swiss Albino Mice

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Abstract

Decremental trend were observed in the total testicular protein. This may be due to random collision of gamma radiation on polypeptide chain of protein molecule causing fragmentation of definitive point i.e "Fragile sites". This process of collision and fragmentation is based on the theory of probability. The fragments thus produced may be small or large and consequently may escape detection by standard biochemical techniques as used in the present studies. The study of gamma radiation thus absorbed may also causes denaturation or coagulation of protein 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 behaviour. Activity of acid phosphatase in the present study was observed to change after exposure to various doses of γ-radiation. 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.radiation induced decrease in LDH may be attributed to 'Switching off of glycolytic pathway for meeting the minimal energy demand of cell types for survival. It seems that testes may use other preferred Krebs cycle intermediate substrate other than lactates.

Keywords: Fertility, Ionized Radiation, Acpase, Alkpase, LDH, Protein.

Introduction

Mammalian testes are an ideal organ to study a variety of cellular process. After administration of Gamma radiation which is most penetrable emanation for mammalian tissues (Ellis, 1970; Grahn et al, 1988; Liu, et al 2006; Khan, 2015; Adikariet al., 2015) causes variety of oscillation in cytoarchitecture, permeability, irritability, conductivity and metabolic status of cell.The extent of such perturbation in many cases appears to be dependent on dose duration relationship. However, this is not always necessarily true due to heterogenecity of tissues and their physiological and biochemical status. Several physical, chemical and biological factors play a deterministic role in radiosensitivity response. Radiation has the ability to cause ionization and formation of free radicals which is suggested to cause cellular injury and genetic lesion (Hawas 2013, Eberhard, et al., 2013). Radiation energy may also cause denaturation of protein. Post radiation 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; Comish, et al. 2014). It also have decremental effect on ribosome number and mRNA synthesis. Such an action would obviously disturb protein synthesis. Several cytopathologies and aberrations in the enzyme-isoenzyme; and enzyme-substrate profile have been described in different somatic tissue type. LDH is one of the key enzyme of the glycolytic cycle which catalyses the reversible reaction lactate↔ pyruvate in the presence of NAD which act as electron acceptor. Elkington et al., (1973), Duowei et al., 2013, showed it is very active in the interstitium and absent in leydig cells, and exhibited relatively attenuated activity in the seminiferous tubules.

The cumulative effect of this action would disturb cell division, maturation, and spermatogenesis and sperm motility. This may mean severe impairment of fertility. Experimental studies have shown that testicular cells are responsive to such physical factors as temperature and radiation. Radiosensetivity of pre-meiotic spermatocytes and differentiating spermatids has been documented by several investigators (Lataillade et al., 1991; Mamina and sheiko 1993 and Kangasniemi et al., 1996, Liu et al., 2006; Khan et al. 2015).

Review of Literature

Radiation induced pathologies have been studied in several placental mammals (Hawas, 2013, Eberhard et al., 2013). Ionized radiation was the first to be recognized as environmental pollutant to affect 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 Benhawy et al., 2015) . Natural background radiation of various forms exists in the biosphere and comes from three well known and studied sources *i.e.*, cosmic rays, living cells and earth crust. Living cells, which have the inherent capability to bio-accumulate and bio-amplify radioactive isotopes from the environment. A variety of radioactive elements such as radium, thorium and uranium are present in the earth's crust and emit α, β, γ-rays. Such radioactive elements are extracted and put to use in various industries, nuclear weapons test explosions, medicine, power generation, agriculture and radio-sterilization (Singh 2011, Waghmare et al., 2013, Zalewska and Suplinska, 2013).

Aims of the Study

The information on these aspects in gonadal tissues is somewhat fractured and, therefore, needs further study to arrive at some common and meaningful conclusion. In the present study with sexually mature Swiss albino mice an attempt has been made to delineate the effect of various doses of gamma radiation on total testicular protein, acid and alkaline phosphatase (AcPase and AlkPase) and lactate dehydrogenase (LDH) vis-à-vis control.

Material and Method

Test Animal

Sexually mature Swiss albino mice weighed 18gm ± 2gm were used as a "model" for the present study to investigate the effect of various doses of gamma radiations on the testes. The mice were maintained on standard rodent chow *ad libitum* access to clean sterilized water. They were kept in mice cages at 26 degree cent. ± 2 deg cent in 12 h dark 12 h day light.

Group 1

Groups 1 served as control, and were sham irradiated.

Group 2

Group 2 were irradiated by 0.1Gy of gamma radiations.

Group 3

Group 3 were irradiated by 0.20Gy of gamma radiations.

Group 4

Group 4 were irradiated by 0.30Gy of gamma radiations all experimental groups and control group were sacrificed after 24 h after giving single dose of irradiation.

These experiments were repeated twice.

Procedure of Radiation

The animals were restrained in position by tying rubber bands around the forelimb and hind limbs. They were exposed to single pulse of various doses of gamma radiation by Cobalt -60 camera .Radiation were applied to the abdominal region where the paired testes were located.

Surgical Process and Preparation of Testicular Homogenate

Mice of control and experimental groups 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 of fascia and blood clots; rinsed several times in chilled physiologic saline (4 deg) .After blotting the tissue the wet weight of each testes were separately recorded on monopan electric balance. Homogenate of testes(100mg/ml) were prepared in normal saline (0.9% w/v) in ice bath in potter Elvehjem homogenizer (for 5 min) .The homogenate were centrifuged at 3000 rpm for 20 min to obtain the subcellular fraction. The supernatant was decanted and utilized for biochemical assay of total protein (T.P) and as per procedure detailed below.

Total protein was estimated according to the method of Henry, *et al.,* (1957) .which is based on the biuret method.

Principal

Tissue protein reacts with copper of biuret reagent in alkaline medium to form a blue purple complex, whose intensity is directly proportional to the protein concentrations.

Protein cu3+/alkaline pH blue colour.

Procedure

1. Sets of three test tubes were labeled as "Test" (T) "Standard"(S) and "Blank" (B).
2. Tissue homogenate (.01ml) was added to "T" test tube.
3. Protein standard (6gm %) was added in "S".
4. Working solution (100ml D.W+ one biuret reagent bottle), was added in all three T, S, B tubes.
5. All were vortexed and thus mixed well; and allowed to stand at room temperature for 10 – 15 min.
6. After 15 min optical density (O.D) of "S" and "T" against the "B" was measured at 550 nm.

Calculation

T.T.P = OD of test/OD of standard × 6 gm %

Alkaline Phosphatase (AlkPase)

AlkPase was estimated by Kind and King's method 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.

Phenyl phosphate $\xrightarrow[\text{pH 10.0}]{AlkPase}$ Phenol + Pi (Disodium hydrogen phosphate)

Phenol + 4-aminoantipyrine $\xrightarrow[\text{OH-1}]{KCN}$ 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 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

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

Acid phosphatase (AcPase)

Acpase in the testicular homogenate was estimated by Kings and Jagathesan's method. (Kingand Jegatheesan 1959)

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.

1. Disodium phenyl phosphatase $\xrightarrow[\text{pH 5.0}]{AC\ pase}$ Phenol + Pi (Disodium hydrogen phosphate)
2. Phenol + 4-AA $\xrightarrow{KCN}$ Orange-red coloured complex. OH^{-1}

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

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

Alkaline Phosphatase (AlkPase)

AlkPase was estimated by Kind and King's method (Kind and King1954).

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.

Phenyl phosphate $\xrightarrow[\text{pH 10.0}]{AlkPase}$ Phenol + Pi (Disodium hydrogen phosphate)

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

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

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

Lactate Dehydrogenase (LDH)

LDH was biochemically quantitated in the testes by using 2-4-DNPH method of King (1959) and Vaishnav (1974).

Principle

LDH catalyses the following reaction

Lactate + NAD $\overset{LDH}{\Longleftrightarrow}$ Pyruvate + NADH products so formed are coupled with 2,4- dinitrophenyl hydrazine (2,4-DNPH) to give the corresponding hydrazone, which gives brown colour in alkaline medium and is measured colorimetrically.

Procedure

Two tubes labelled as 'Control' (C) and 'Test' (T) were set up in pairs. Buffer lactate substrate pH 10 (0.5 ml) was added to both tubes. Distilled water (0.1 ml) and tissue homogenate (0.05 ml) were added to 'C' and 'T' respectively. They were vortexed and incubated at 37 °C for 5 min. Then NAD solution (0.1 ml) was added to test 'T' and again incubated at 37°C for 15 min. To this colour reagent DNPH (0.5 ml) was added while in the test tube 'C' tissue homogenate (0.05 ml) was added and vortexed. Incubation was carried on for 15 min at 37°C. Finally 4M NaOH (0.5 ml) was added to both tubes and absorbency was

measured at 440 nm. The standard curve was prepared by changing the concentration of 1.0 M working pyruvate standard, NADH, buffer, and lactate substrate (pH 10.0) in the total assay system containing NAD, DNPH colour reagent and 4 M NaOH incubated for 15 min at 37°C.

Calculation

Net O.D. of the Test = O.D. Test — O.D. Control

The enzyme activity was computed and represent in the IU (international unit)

Results and Discussion

In total protein (Table) Decremental trend were observed. This may be due to random collision of gamma radiation on polypeptide chain of protein molecule causing fragmentation of definitive point i.e "Fragile sites". This process of collision and fragmentation is based on the theory of probability. The fragments thus produced may be small or large and consequently may escape detection by standard biochemical techniques as used in the present studies. The study of gamma radiation thus absorbed may also causes denaturation or coagulation of protein. Post irradiation may also cause decrease in the number of ribosomes the "sites of protein synthesis". Such an action evidently would disturb the process of translation i.e., protein synthesis itself this suggestion corroborate the *vitro and vivo* findings of other investigators (Eberhard et al, 2013).

Thus the cumulative effect of gamma radiation may disturb the synthesis of protein either by disturbing RNA, ribosome's, or protein itself or in extreme situation the entire mechanism is "knocked out". The large changes seen in response to 0.05Gy appear to be due to high vulnerability of testicular cells which may be in a state of division or differentiation. The damage caused to such cells would lead to their lyses and consequent leaching out of cellular contents (Liu et al., 2006). This may have enhanced the T. P. estimates. On the other hand the differential results with higher doses by gamma radiations may mean a differential response of testicular cell type or the random collision between gamma ray and cellular protein may have failed to afflict more damage. Thus necrotic and lethal effects of such radiation do not appear to be very high.

No comparisons are feasible due to lack of information on this aspect. However, electrophoretic studies of Karlish & Kempner (1984), Beauregard *et al.,* (1987) indicate that this may be logical explanation of these results. Lack of adequate protein turnover would have a deleterious effect on spermatogenesis and sperm motility (Chinoy et al., 1980, Comish et al. 2014).This agrees well with the result of present studies.

Thus, the results of the present studies clearly indicate that various doses of gamma radiation detrimentally affect the biochemical melieu of testes. This is exhibited by estimation of total testicular protein which shows a following dose related pattern.

Total testicular Alkpase (table) 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 0.05Gy and 0.1 Gy to be 24.13% and 21.8% respectively vis-a-vis control. Alkpase is an 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 (Kalplan andMarshall 1972, Gaud *et al.,* 1982), 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. Spermatids are known to be relatively radioresistant. (Lataillade *et al*., 1991, Kangasniemi *el al.,* 1996). However, if cells are in premature stage extent of the damage would be large. The quantitative estimates of total **testicular Acpase (table)** in the testes of male Swiss albino mice challenged by various dose of y-radiation were observed to increase in response to challenge by 0.5 Gy and 0.15 Gy. 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 (Ravindra et al., 2009). Lysosomes are implicated in intracellular protein digestion and can also cause autophagic digestion of cellular organelles or the cell itself i.e., autolysis (de Duve 1959 and Novikoff, 1961.Radiation-induced cellular degradation of tissue damage was shown (Hugon and Borgers, 1965, Wills and Wilkinson,1966, Dhawan et al.,1996)

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 1979. The increment in the activity of AcPase in mammalian tissue was reported by Ravinder et al., 2009, Kroll Nipper, 1987, Samarth et al.,2001 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.

Nehru et al.1991 reported a significant decrease in the activity of Acpase at 2.5 Gy and 10 Gy 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.

Lactate dehydrogenase (LDH) (Table) concentration in the testicular homogenates of control and experimental groups manifested their minimal and maximum values vis-a-vis control (860 units) as shown in the table. The activity of LDH testes irradiated by 0.05 Gy was 822.5 units; by 0.1 Gy, 819.8 units; by 0.15 Gy, 827.3 units by 0.2 Gy and by 0.25 Gy these values were 681.35 unit and 730.5 units respectively. These values are less than the control. Testicular LDH activity was maximally decreased on exposure to 0.20 Gy and minimal decrease was observed in case of 0.15 Gy of irradiation γ-radiation induced decrease in testicular LDH of mice vis-a-vis control. Pant *et al.,* (1995) reported increase in testicular LDH level by oral administration of carbofuran. However, reduction in the kinetics of spermatogenesis, and enzyme activity after ionizing irradiation was observed by Ivanitskaiaet al.,(1991).

A variety of dehydrogenesis are involved in metabolic pathways that are critical in the bioenergetics of cells. LDH is an important enzyme of the glycolytic cycle which catalyses the reverse transformation of pyruvate to lactate in the presence of NAD which acts as a co-factor (Markert and Moller 1959).

Table: Quantitative Shifts in the Amount of Total Protein, Acid Phosphatase, Alkaline Phosphatase and LDH in the Testis of Swiss Albino Mice Exposed To Various Doses of ^{60}CO-Γ-Rays

Dose Gy/ min	Total protein (gm%)	Acpase (KA Unit)	Alkpase (KA Unit)	LDH (in IU)
Control	162.333±02.809	6.103±0.272	22.837±0.408	860±10
0.05	134.833±10.900	8.553±0.388	28.343±0.596	822.50±2.50
0.10	150.933±05.382	4.737±0.135	27.817±0.726	819.50±1.50
0.15	142.133±02.824	10.830±0.217	12.817±0.457	827.30±28.30
0.20	149.933±01.097	6.090±0.365	17.343±0.337	681.35±11.05
0.25	149.233±03.886	4.673±0.340	16.283±0.222	730.50±5.50

Conclusion

The quantitative estimation of proteins and other enzymes i.e., **Acpase,** Alkpase,LDH after giving various doses of gamma radiations altered and its cumulative effects would disturb maturation, spermatogenesis, and sperm motility. This may mean severe impairment of fertility.

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