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Effect of Gamma Radiation on Total Testicular Protein of Swiss Albino Mice

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Abstract

The amount of total protein in the testes of irradiated mice displayed decremental trend vis –a- vis control. T P of control was 162.33 mg/dl. While in case of irradiated groups the corresponding values for 0.1Gy it was 150.93 mg/ml; and for 0.2 Gy and 0.30 Gy the corresponding values were 149.933 mg/ml, and 149.23 mg/ml respectively. Which shows 6.83%, 7.45% and 7.88% lower than the control (which was considered as 100%).

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 (J Infrared Milli Terahz Waves 2011)

Keywords: Ionized Radiation, Total Protein, Fertility, Mammalian Testes.

Introduction

Mammalian testes represent a intricate association of heterogenous cell population whose primary exocrine function is to produce spermatozoa; and endocrine function is to synthesize and release a variety of androgens. These functions are distinctly compartmentalized i.e., spermatogenesis in the semineferous tubules and androgenesis in the leydig cells. A sustained generations of precursors via enzymatic intervention occurs in both processes .However in the former this entails the formation of stage specific protein during spermatogenesis. The metabolic status of germ cells, endocrine cells (Leydig) and somatic cells (Sertoli) of the testes is known to undergo cyclic changes that coincides with the cycles of the seminiferous epithelium and hormone production. The testis is considered as one of the most radiosensitive organ of the mammals (Ellis, 1970; Grahn and Carnes, 1988; Liu et al.,2006 Khan et al.,2015. Considerable information is available on the gamma radiation induced histo- and cyto-pathologies of mammalian testes. It 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. However, studies on alteration in the biochemical milieu have received relatively less attention. The literature on dose and duration related changes in the testicular protein (T.P) is relatively scant and fragmented.

The present investigations were therefore, carried out to monitor the biochemical changes in the testes of Swiss albino mice challenged by single pulse of gamma radiation.

Aims of the Study

Mammalian testes is an ideal organ to study a variety of cellular processes i.e. cell division, growth, differentiation and maturation. Radiation induced damage to testis 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 (Wilmink & Grundt 2011). This threat is real, since all cell and organismshave the inherent ability to bioamplification e.g mutation, cancer, tereta formation and cytogenetic aberrations (Eberhard et al. 2013; Comish et al., 2014). The

information on these aspects in gonadal 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.

In the present study with sexually mature Swiss albino mice has attempt has been made to delineate the effect of various doses of gamma radiations on the total testicular protein.

Material and Method

Test Animal

Sexually mature Swiss albino mice weighted 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 are 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

Served as control, and were sham irradiated.

Group 2

were irradiated by 0.1Gy of gamma radiations

Group 3

Were irradiated by 0.20Gy of gamma radiations

Group 4

Were irradiated by 0.30Gy of gamma radiations All experimental groups and control group 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 react 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 %

Results and Discussion

Total Protein (T P)

The amount of total protein in the testes of irradiated mice displayed decremental trend vis –a-vis control. T P of control was 162.33 mg/dl. While in case of irradiated groups the corresponding values for 0.1Gy it was 150.93 mg/ml; and for0.2Gy and 0.30Gy the corresponding values were 149.93 mg/ml and 149.23 mg/ml respectively which shows 6.83%; 7.45% and 7.88% lower than the control (which was considered as 100%).

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.

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 his suggestion corroborate the *vitro and vivo* findings of other investigators (*vide infra*) (Eberhard et al, 2013).

Thus the cumulative effect of gamma radiation may disturb the synthesis of protein either by disturbing RNA, ribosomes, or protein itself or in extreme

situation the entire mechanism is "knocked out". The large changes seen in response to 0.10Gy 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 lysis and consequent leaching out of cellular contents (Hadaf,,2003, 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 effect 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

Durationofsacrifices afterradiation(hour)	Dose in Gy/0.30min	Total protein (gm %)
Control	---------	162.333±02.809
24	0.10	150.933±05.382
24	0.20	149.933±01.097
24	0.30	149.233±03.886

Conclusion

The present study provides base line and significant information on the effect of various doses of gamma radiation on the testicular protein, and its cumulative effects of the action would disturb maturation, spermatogenesis, and sperm motility. This may mean severe impairment of fertility. The result is supported by studies such as Comish et al (2014).

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