Effect of Beta Radiation on Extraocular Muscles

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Abstract: Purpose: To evaluate the effect of different Beta radiation doses on frogs extraocular muscles. **Methods:** 50 frogs of species Rana Ridibunda were used in this study, they were divided into 5 groups, every group was treated with a different dose of radiation, and the first group was taken as control. **Results:** The estimation of soluble protein content in extraocular muscles of Beta radiated eyes showed a gradual decrease with the increase of dose. **Conclusion:** Significant changes in extraocular muscles were detected with the increase of Beta radiation dose.
[Mohamed A. Marzouk, Hossam E. Sayed, Ayman A. Shoman, Hisham A. Hashim. **Effect of Beta Radiation on Extraocular Muscles.** Journal of American Science 2010;6(12):1028-1033]. (ISSN: 1545-1003). http://www.americanscience.org.

Keywords: Effect; Beta Radiation; Extraocular; Muscle

Introduction:

Almost infinite variety of atomic, intra, and intermolecular changes will occur when living tissue is subjected to ionizing radiation. Some of these changes may be the goal of radiotherapy, while, of course, the others are considered to be harmful or even lethal changes [(1)].

Living tissue absorb the ionizing radiation through two mechanisms: ionization, and excitation depending on the energy of that radiation; if its energy is high enough, some molecules may be ionized causing direct damage to the other neighboring molecules, or even the far molecules through the formation of free radicals (indirect effect). If the energy of the radiation is not enough to ionize the molecules, it may cause its excitation to higher energy levels, therefore disturbing the normal balance of the organism [(2)].

Beta radiation is one of the most abundant radiation types being used in ophthalmology, it has a small penetrating power and that is to be highly considered while dealing with the eye which is one of the most radiosensitive organs of the body [(2)].

Beta radiation has been used for ocular radiotherapy since the forties of this century. Postoperative treatment of pterygium[(3)] ,and more recently primary pterygium with no prior surgical excision[(4)], may be the most pronounced application of Beta radiation in ophthalmic therapy, however, there are some other situations at which Beta rays are used successfully, like non malignant lesions of the eye lids, corneal scars, corneal vascularization, vernal conjunctivitis, superficial or deep keratitis[(5)], and in postoperative treatment of total and subtotal symblepharons[(6)] .It's also being applied in some centers now, with more advanced techniques, for the treatment of conjunctival squamous cell carcinoma , choroidal and ciliary body melanomas[(7)], also in controlling the elevated IOP by its effect on the ciliary body and wound healing alteration [(8)].

Many workers have early noticed complications due to Beta radiation such as telangiectasis of the conjunctiva, keratinization of the conjunctival epithelium that led to punctate keratitis resulted in severe lacrimation. Other late effects of beta radiation were also reported such as scleral thinning, scleral ulcers, atrophy of the sclera, superficial punctate keratitis, corneal vascularization, corneal scarring, perforation, iritis, iris atrophy, iridocyclytis, cataract, ptosis, glaucoma, and endophthalmitis[(5,9,10.11)].

These side effects became will known later and the strategies of Beta radiotherapists had to consider the dose-effect on such symptoms, so, many workers began to use smaller doses to overcome such complications, and also set dose-fraction systems to optimize therapy and minimize the side effects [(12)].

Such dose-fraction systems are being established and used in the Beta radiotherapy centers; and for the current study, the applied dose fractionation scheme for the experimental animals is that used for human application in the postoperative treatment of pterygium in the dept. of radiotherapy at the Research Inst. Of Ophthalmology, Cairo-Egypt. According to this scheme (which complies with the findings of Bahrassa et al, 1983; and El Dessoki, 1990), patients receive a total accumulated dose of 24 Gy (32 Gy in some cases) of beta radiation in three (four) divided sessions in a week[(3,13)] .

Materials And Methods:

Fifty frogs of species Rana Ridibunda, of average weight 74 gm., were used in the present study. Frogs were divided into 5 groups, each of which consist of 10 animals and labeled A, B, C, D, and E. Group A was taken as the control (untreated) group. The other 4 groups were treated with different doses of radiation as follows: Group B received 8 Gy, group C received 16 Gy, group D received 24 Gy, and group E received 32 Gy.

The species Rana Ridibunda was chosen on the basis of its larger size and stronger musculature compared to the other available species. Eyes of the frog Rana Radibunda have close structure to human eyes and similar extraocular muscles[(14)].

The radiation source used in the study was a Strontium-90/Yttrium-90 source, which is contained in the eye applicator (SIA.20- Amersham, UK) found at the Research Institute of Ophthalmology, Giza, Egypt .

For all animals, the right eye was the treated eye. After irradiation, animals were left alive for up to 30 days from the last session.

For the extrusion of the extraocular muscles, the animal was slaughtered and the head was separated, the buccal cavity was opened and the lower jaw & tongue were removed and the upper membrane was peeled to uncover the eye globe and extraocular muscles. The extraocular muscles were excised from the globe and then weighted to determine the wet weight.

Due to the small size of the muscles from a single animal, a pooled sample of muscles from the individuals of each group was considered to increase the sample size. Enough impact sample of each group was preserved in formalin solution for the histological examination, and the rest of the pooled sample was then treated as described by Baldwin et al., (1955) [(15)].

We did use the following methods in detecting changes in extraocular Muscles:

1-Quantification of soluble protein content by Lowry method .

2-Determination of protein pattern by Gel Electrophoresis .

3-Spectrophotometric measurements.

4-Histopathological examination .

Results:

During the radiation process, the Beta applicator (type SIA 20- Amersham) was placed in contact with the cornea of the frog eye simulating the therapeutic technique used for human treatment.

This means that the cornea dose can be considered to be the same as the surface dose of the applicator[(16)]. Accordingly all Beta radiation doses mentioned in this study refer to the cornea dose, while that of the extraocular muscles is only 2-4% of the cornea dose which can be estimated from the dose curves of the strontium 90 Beta applicator, and this applies to all results[(16)].

Quantification of soluble protein content by Lowry method:

The soluble protein content of the treated extraocular muscles decreased gradually from 90% of the control and group B, (8Gy cornea dose) to 55% in group E, (32Gy cornea dose).

The decrease of soluble protein content of extraocular muscles as a result of Beta radiation indicates that some type of destructive action has occurred. Study of the protein content only is not enough to explain the possible changes. More explanations can be obtained if the different samples were subjected to electrophoresis. (Fig. 1 A).

Determination of muscle protein patterns by Gel Electrophoresis:

It can be shown from the separation pattern that the bands showed an apparent difference in the separation bands of Actin, Beta-actinin, and Tropomyosin of the last two treated groups (that received 24, and 32Gy Beta radiation corneal dose), the bands of Beta-actinin and Tropomyosin (bands 1 and 2) broadened and diffused together, while the other two bands of Actin and Myosin light chain (bands 3 and 4) became much more broadened which implies an alteration of the protein structure that may be due to the aggregation of some protein fragments in the samples. A structural deformation means malfunctioning of the affected muscle which needs further investigation in a future study. (Fig. 1 B).

Spectrophotometric measurements :

A general trend of higher absorbance values for the first two groups B&C (8 & 16 Gy corneal dose) compared to control, while the last two groups D&E (24 & 32 Gy corneal dose) which received higher radiation dosed showed lower absorbance values compared to control indicating some form of protein damage. (Fig. 1 C).

Histopathological examination:

-In group B (8 Gy):

The normal striation of muscles is unaffected, with some fatty infiltrations.

-In group C (16 Gy):

The presence of fatty infiltrations became more apparent, and the affinity to the haematotoxylin stain seems to be decreased.

-In group D (24 Gy):

The affinity to the haematotoxylin stain showed more decrease, and the muscle striation is disrupted compared to normal muscles and the myofibers showed some splitting.

-In group E (32 Gy):

The affinity to the haematotoxylin stain is much more decreased and the normal striation of myofibers is extensively disrupted. The myofibers showed more splitting and many myofibers showed the absence of nuclei (Fig. 2).

Discussion:

To our humbled knowledge this is the first study on the interaction of Beta radiation with the extraocular muscles.

Although the amount of Beta dose of the extraocular muscles of the frog was only 2-4% of the cornea dose, many changes could be found due to such doses.

The estimation of soluble protein content of extraocular muscles in Beta radiated eyes showed a gradual decrease with the increase of dose. This implies change in the nature of proteins of treated samples which increases with increase of applied dose.

Data from electrophoretic measurements also confirmed the same conclusion, where other molecular weights appeared in the separation pattern indicating the appearance of new protein fragments.

Spectrophotometric measurements focused on the effect of different Beta radiation doses on peptide bonds and nucleic acids which showed that there are different degrees of damage with different doses, which means that proteins were exposed to some type of damage which produced some new protein fragments which agrees with the previous conclusions.

The direct effect of Beta radiation can't be the only reason for such changes. The effect of Bremsstrahlung radiation can be considered to be an important factor in the appearance of many side effects of Beta radiation due to its higher penetration in tissue so that it can cause such distant changes.

Bremsstrahlung are X-rays that are emitted when high-speed charged particles suffer rapid acceleration. When a β particle passes close to a nucleus, the strong attractive Coulomb force causes the β particle to deviate sharply far from its original path. The change in direction is due to radial acceleration, and the β particle, in accordance with classical theory, loses energy by electromagnetic radiation at a rate proportional to the square of the acceleration. This means that the Bremsstrahlung photons have a continuous energy disruption that ranges downward from a theoretical maximum equal to the kinetic energy of the β particle. The likelihood of Bremsstrahlung production increases with the atomic number of the absorber [17].

Another factor to be considered, that is the free radicals which are formed in the aqueous medium within the tissue (which is abundant in the structure of the eye). Radiation-produced free radicals affect other molecules at a distance as they can transfer their "extra" electron to a nearby molecule, which in turn, may pass it on. The electron can be passed through a succession of molecules forming many free radicals. This chain reaction may occur in a large number of molecules and results in critical changes in organic molecules. Similarly, a free radical may "snatch" an electron from a neighbor, which then becomes a free radical. A chain of electron "snatchings" may ensue an organic molecule to be critically changed in the process. Therefore, the radiation damage that occurred in the β irradiated samples can be thought to be a result of the free-radical interactions [17].

Conclusion:

Therapeutic doses of Beta radiation on the eye can harmfully affect the structure and function of extraocular muscles despite its short penetrating range in tissues, and the risk to benefit ratio should be considered during its use.

We are in need for a future human studies on the extraocular muscles regarding it's balance and function after therapeutic Beta radiation.

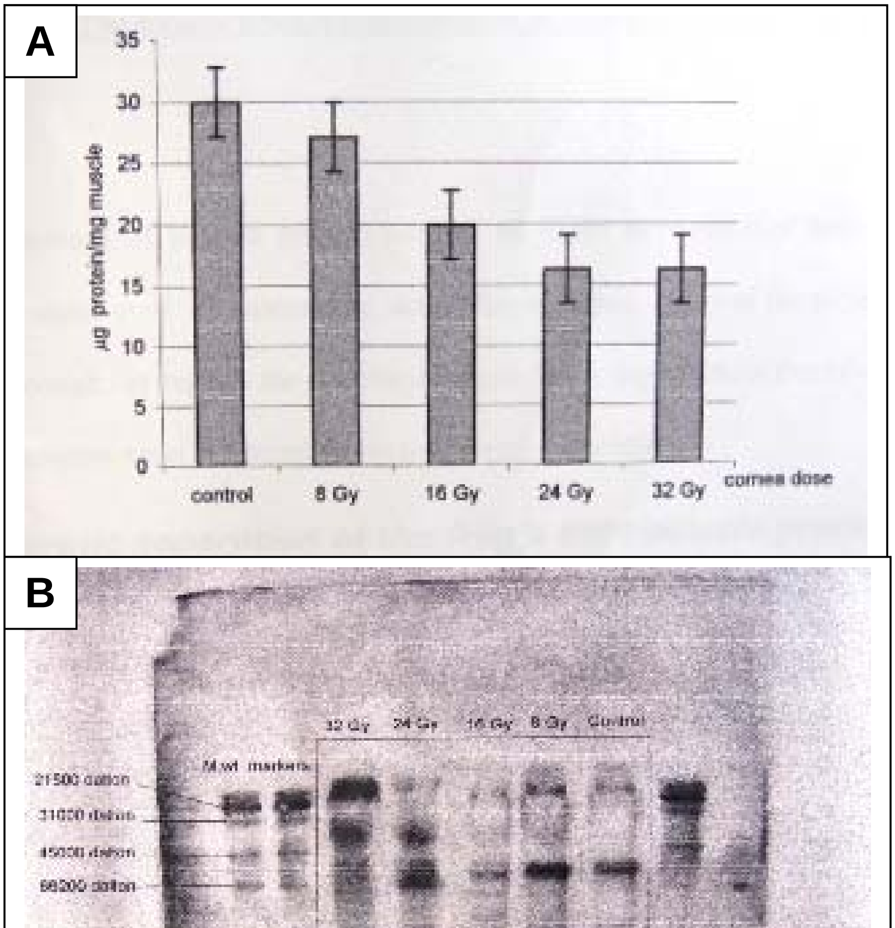
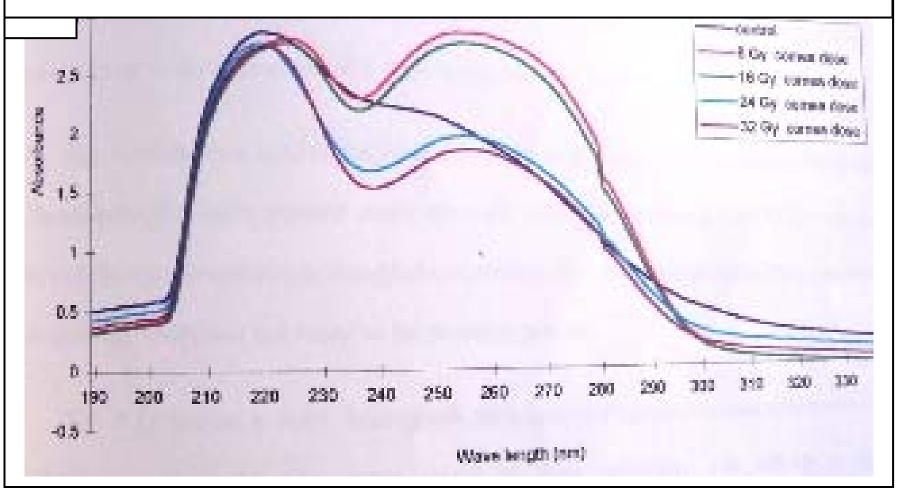


Fig. 1
Changes between control and different groups in
A- Protein content **B**- Gel Electrophoresis
C- Spectrophotometery



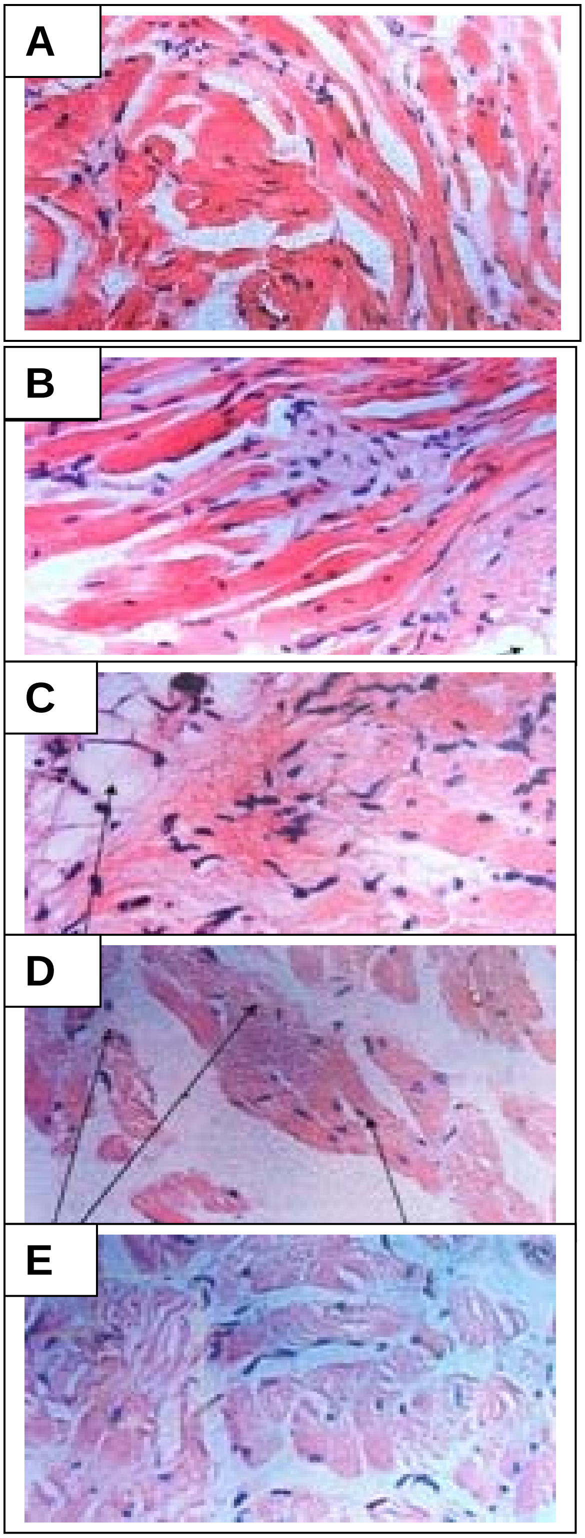


Fig. 2. Histopathological changes in different groups
A,B,C,D&E

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10/24/2010