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Research Article

COMPARATIVE STUDY BETWEEN TOPICAL APPLICATIONS OF LIPOSOMALLY ENTRAPPED DNA REPAIR ENZYMES AND THYMIDINE DINUCLEOTIDES AS RADIOPROTECTORS

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ABSTRACT

The delivery of active agents to the skin by liposome carriers is of great interest during the last three decades, based on their potential to enclose various types of biological materials and to deliver them to diverse cell types. Purpose: is to compare between topical application of DNA repair enzymes and thymidine dinucleotide which is a photoprotective agent against non-ionizing radiation through induction of DNA repair. Materials and Methods: thirty six Albino rats were treated by either thymidine dinucleotides or liposomally entrapped DNA repair enzymes topically, 24 hours after they were irradiated with Cobalt-60(60Co) gamma radiation with different doses (0.5 and 3Gy), and they were evaluated with histolopathologically by H&E stain and computerized image analyzer using Masson's trichrome stain. Results: Gamma radiation produced epidermal thinning and dermal inflammatory cells together with collagen fragmentation and clumping in a dose-like manner. Thymidine dinucleotides gave a better response with both (0.5& 3 Gy) than liposomally entrapped DNA repair enzymes, the epidermis was preserved of with no inflammatory cells and also it maintained the normal architecture of collagen bundles. Conclusion: The effects of gamma radiation on the skin could be minimized by the use of certain dinucleotides.

Keywords: Gamma radiation/ thymidine dinucleotide/ liposomally entrapped DNA repair enzymes.

INTRODUCTION

The ideal radioprotective agent that has the ability to block electromagnetic photons from entering the skin enhances the skin's ability to repair damage from any absorbed photons, and yet permit tanning. Sunscreens effectively absorb or reflect sunlight, but do not alter the skin's innate repair capacity and do not have any effect on ionizing radiation.

Human skin is a complex organ covering the body's internal systems and organs. It is the body's external interface with the environment, and is considered as a target organ for pollution and also the site of significant absorption of environmental pollutants. Skin cancers are increasing nowadays and sunscreens are apparently underused and misused[1]. Many studies had been done on various radioprotectors including; Amifostine, caffine, troxerutin and melanin[2-5] to act on cellular DNA against the deleterious effects of gamma-radiation.

In 2012 Goukassian and his colleagues reported that thymidine dinucleotides (pTpT) induced DNA repair to newborn mice and reduced and delayed the development of UV-induced melanomas. On the other hand, liposome encapsulation of T4 endonuclease V represents an approach that shuttles enzymes across human stratum corneum and introduces biologically active proteins into living epidermis[6]. Furthermore, liposomes are considered, as vehicles for topical drug delivery that may be superior to conventional preparations, which suggests that only a compromised epidermal barrier enables intact liposomes to penetrate the skin. It was originally isolated from Escherichia coli infected with T4 bacteriophage. It has been shown to repair ultraviolet (UV)-induced cyclobutane pyrimidine dimers in DNA, which, when unrepaired, contribute to mutations that result in actinic keratoses and non-melanoma skin cancers (NMSC). This is a particular concern in patients with genetic defects in their DNA repair systems, especially those with xeroderma pigmentosum (XP)[7].

The principal epidemiologic studies of ionizing radiation and skin cancer have all shown that radiation causes basal cell carcinoma but have not found dose-related excess of squamous cell carcinoma or malignant melanoma. The Japanese atomic bomb study indicated that doses of radiation under about 1 Gy confer less risk per unit dose than higher doses do[8]. Significant doses of ionizing radiation produce acute skin reaction in human called acute radiodermatitis, which are dose-dependent and reflect damage to the germinative cells of the epidermis and to the cutaneous vasculature. When the dose is about 3.8Gy, it is characterized by erythema, edema, slight burning and pruritic sensation, healing usually occurs within 2-14 days. However, temporary epilation may be also associated, and hair regrowth occurs after 4-12 weeks. Moreover, when the exposure exceeds 10Gy the epidermis exfoliates leaving a denuded dermis, with punched out painful ulcer, with no granulation tissue formation.

When the ICRP made its recommendations in 1977 for dose limits there was no appreciation of the importance of the interaction of ultraviolet radiation (UVR) and X-rays. Both clinical and experimental data showed that the risk of ionizing-radiation-induced cancer is significantly increased by subsequent exposures to UVR. Therefore, risks for sun-exposed areas of skin differ from those that are shielded. The risk estimate for skin cancer is very dependent on the selection of the skin site, the total risks for both UVR exposed and shielded skin is about twice that of shielded skin in non exposed skin areas[9]. According to the existing standard, maximal dose to the skin over the career may not exceed 1200 cSv. However, there is high probability of pathology including malignant tumor in delayed period after exposure to 1200 cSv. The maximal dose to the skin over the space career should not be higher than 600 cSv[10].

MATERIALS AND METHODS

Forty-two albino rats (Rattus rattus) were included in the current study. Thirty six rats were irradiated (whole body) using cobalt-60 (Gamma-cell 220), Atomic Energy of Canada Limited, installed at Radioisotope Department of the Egyptian Atomic Energy Authority, Cairo-Egypt. This study obtained ethical clearance number from Institutional ethical committee. Cobalt-60 is a radioactive isotope of Cobalt, which the physical ½ life is 5.27 years. It decays by beta particle and gamma ray emission. This source provided an average exposure rate of 1 Gy/min in the center of the cage of the machine of irradiation.

Punch skin biopsy 6 mm was taken from each rat. They were divided into: Group I: without painting. Group II: painted with Liposomally Entrapped DNA Repair Enzymes, specific plant extract from the nopal cactus "Nopasome" (Ateia AG-Austria). Group III: painted with Thymidine Dinucleotides (pTpT) (Midland Certified Reagent Company- USA. Group IV: normal rats without painting and irradiation.

Each of the above 3 groups was subdivided into 2 subgroups (I/0.5 and I/3), (II/0.5 and II/3) and (III/0.5 and III/3), each of them were 6 rats. Twenty four hours after application of topical pTpT and liposomally entrapped DNA repair enzymes, irradiation of 0.5 Gy gamma rays using cobalt-60 to subgroups I/0.5, II/0.5 and III/0.5 ; 3 Gy to subgroups I/3, II/3 and III/3.

Skin biopsies were taken from each rat daily after 24 hours of irradiation on the 1st, 2nd, 3rd and 4th day. Punch skin biopsy 6 mm was taken and put in buffered formalin 10% then processing with paraffin, and then cut on a rotary microtome into sections 5-7μm thick and stained with Haematoxlin and Eosin stains (H & E), sections were then examined histopathologically. Masson's trichrome stain was also done in order to demonstrate collagen changes in the rat skin dermis. Evaluations of skin biopsies were done and a comparative study was done on skin biopsies between treated subgroups against control.

Image analysis: The data were obtained using Leica Qwin 500 image analyzer computer system (England). The image analyzer consisted of a colored video camera, colored monitor, hard disc of IBM personal computer connected to the microscope, and controlled by Leica Qwin 500 software. The image analyzer was first calibrated automatically to convert the measurement units (pixels) produced by the image analyzer program into actual micrometer units. The area % of collagen fibers in dermis was measured using the color detect menu. The blue staining of collagen by Masson's trichrome was detected and masked by a red binary color. These red areas were measured and their area % in relation to the standard measuring frame of a standard area equal to $29524.44\mu m^2$. Using the measuring field menu the area, area % and standard measuring frame of a standard area equal to $29524.44\mu m^2$ were chosen from the parameters. Ten fields in each specimen were measured and the mean values were obtained using an objective lens of magnification 20, i.e. a total magnification of 200. Using the interactive measuring menu, the epidermal thickness was measured by obtaining 10 readings in each specimen at magnification 200.
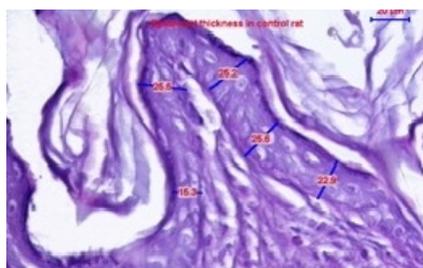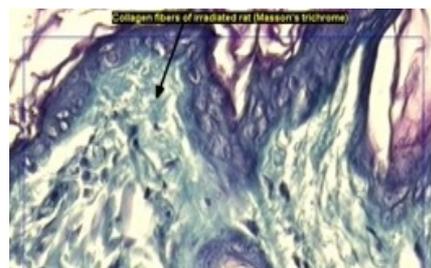

Figure 1: Shows normal epidermis of albino rat skin
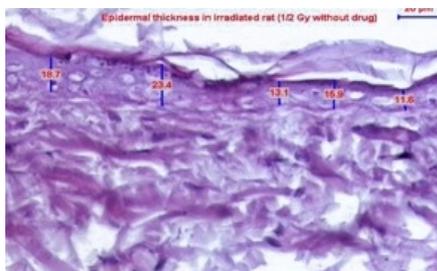

Figure 2: Shows Epidermal thinning 4 days after irradiation

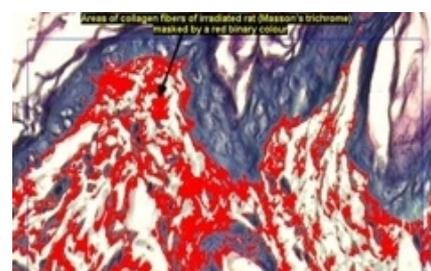**Figure 3: Shows dermis stained by Masson's Stain**

Figure 4: Shows collagen areas masked by red binary color

Table 1: Epidermal thickness

Groups	Rat1	Rat 2	Rat 3	Rat 4	Rat 5	Rat 6	Mean	t-test
Control	22.141± 6.9	22.276±3.18	16.877±4.2	25.163±8.96	25.14±3.91	27.115±8.77	23.119	Control
Group III/0.5	35.18±4.45	34.225±7	35.605±10.1	34.08±4.98	37.314±10.0	35.281±4.45	35.281***	0.0002556
Group II/0.5	34.18±4.33	35.225±44	34.605±13.1	35.08±4.98	36.314±8.0	36.281±3.45	35.053***	0.0002149
Group I/0.5	22.002±5.17	18.543±3.44	16.284±3.5	24.602±2.41	17.57±2.83	19.37±1.83	19.729*	0.0331761
Group III/3	28.235±6.56	21.247±2.93	17.484±4.1	25.373±4.42	25.367±4.73	26.626±5.57	29.555**	0.002105
Group II/3	28.235±6.56	21.247±2.93	14.484±4.1	20.373±4.42	23.367±4.73	21.626±5.57	21.897	0.3957643
Group I/3	14.92±2.89	16.572±1.76	13.756±1.88	12.423±1.7	14.358±3.93	14.977±1.65	14.501**	0.0014013

*/ **/*** are the significant differences at t-test < 0.05, < 0.01, < 0.001 respectively

Table 2: Collagen percentage area in the rat skin dermis

	Rat1	Rat 2	Rat 3	Rat 4	Rat 5	Rat 6	Mean	t-test
Control	39.1±2.4	41.05±3.37	42.7±3.3	40.05±2	38.9±3.3	38.3±3.3	40.05	Control
Group III/0.5	29.023±4.3	34.364±2.9	29.408±5	26.719±4	33.484±6	30.6±2.5	30.6***	0.0002408
Group II/0.5	28.043±2.6	35.364±3.4	28.408±7	27.719±4	32.484±4	31.4±1.5	31.3***	0.0002145
Group I/0.5	27.537±3.9	26.362±2.7	26.999±3	27.712±3	28.176±2	21.272±3	26.34***	0.0002143
Group III/3	24.3±3.03	21.169±2.32	25.703±2	31.287±6	35.358±8	34.419±5	28.706**	0.0024798
Group II/3	28.023±4.3	35.364±2.9	28.988±5	27.659±4	32.544±6	31.6±2.5	29.63***	0.0003456
Group I/3	21.69±5.2	21.48±5.318	31.35±3.3	32.18±3.7	17.32±3	24.79±2.5	24.79***	0.0007223

*/ **/ *** are the significant differences at t-test< 0.05, < 0.01, < 0.001 respectively

RESULTS

The dose of radiation exposure was entirely gamma in the current study; beta radiation coming from cobalt 60 is absorbed within 0.1 mm of water equivalent. Rat skin was affected by gamma radiation and it was found that biopsies taken in first day and second day of irradiation were almost similar and did not differ from the control. However, skin biopsies taken on the fourth day of irradiation showed a highly significant variation. Skin irradiation with single dose of 0.5 and 3 Gy without the use of either pTpT or liposomally entrapped DNA repair enzymes induced on the fourth day of irradiation epidermal thinning, flattening of the rete ridges together with presence of large number of langerhans cells and many pyknotic nuclei (fig.1& 2). A significant epidermal thinning was also noticed with 0.5 Gy and 3 Gy irradiation of about 14.6% and 37.2% respectively on the fourth day in group I/0.5 & I/3 (table.1). On the other hand, when pTpT and liposomally entrapped DNA repair enzymes were added, spongiosis (edema of the epidermis) together with liquifactive degeneration of the basal cell layer were demonstrated. Moreover, a highly significant increase in the epidermal thickness was also detected in II/0.5 and III/0.5 groups of about 52.6% compared to control. Moreover, both group III/3 showed a significant epidermal, while it was insignificant II/3 when compared with the control.

On the other hand, when rat skin dermis was examined; gamma irradiation induced fragmentation and clumping of collagen fibers and dilated blood vessels together with intravascular thrombosis. However, when liposomally entrapped DNA repair enzymes and pTpT were applied as in groups II and III, collagen fibers were healthy with minimal affection. Masson's trichrome stain demonstrated collagen as blue bundles which was stained red by the computer of image analyzer in order to calculate the collagen density in certain areas within the dermis (fig.3 &4). It was found that irradiation lowered collagen density significantly in a dose like manner by 34.2% and 38.1% with groups I/0.5 and I/3 respectively. While when liposomally entrapped DNA repair enzymes and pTpT were added, the collagen density was less diminished than the control to by 23.5% and 28.3% respectively as in groups II/0.5 and II/3 as well as III/0.5 and III/3 (table.2).

DISCUSSION

The response of the skin to ionizing radiation has important implications both for the treatment of malignant disease by radiation and for radiological protection. Acute radiation damage to the skin is primarily a consequence of changes in the epidermis; the timing compare the peak of the reaction is related to the kinetic organization of this layer. Recovery of the epidermis occurs as a result of the proliferation of surviving clonogenic basal cells within the irradiated area. The presence of clonogenic cells in the canal of the hair follicle is important, particularly after non-uniform irradiation from intermediate energy beta-emitters. The migration of viable cells from the edges of the irradiated site is also significant when small areas of skin are irradiated[11].

Epidermal permeability barrier function is impaired in cases of radiation dermatitis. The functional damage to the stratum corneum induced by ionizing radiation occurs with a delayed course, starting within a mean period of 11 days and reaching maximal values after a mean period of 27 days (range, 13-75 days)[12]. Moreover, Goukassian et al., 2002 reported that pTpT treated skin of all mice showed spongiosis and sunburn cells, which was prominent in Xeroderma pigmentosa diseased mice, consistent with compensatory hyperplasia[13].

The current study confirmed that presence of spongiosis, which increased the thickening of epidermis by 52.6% in the fourth day of irradiation when pTpT was previously applied with 0.5 Gy. However, gamma irradiation seems to have a prominent effect on the epidermis as it result in significant epidermal thinning with 0.5 Gy and 3 Gy irradiation of about 14.6% and 37.2% . Moreover, large number of langerhans cells was present in irradiated rat skin and disappeared with topical treatment. Studies in the pig skin, showed that postregenerative phase of hyperplasia reflect a temporary overshoot of cell density above control levels; a subsequent decrease in hyperplasia indicates feedback control of cellular proliferation[14].

Collagen is known to constitute 70% of dry skin mass, which is either collagen type I, III and VI. Collagen type I, which comprises 80% of total collagen, decreases by 59% in irradiated skin depending with extent of exposure. UVR is known to cause collagen damage through up regulation of several types of collagen-degrading enzymes called Metalloproteinases[15].

Ionizing radiation is known to cause fattening of the rete ridges and accumulation of edema fluids in the dermis together with fragmentation and clumping of dermal collagen known as radiation dermatitis. Dilatation of dermal blood vessels may also give rise to telangiectasia[16]. The results of this study confirmed these findings especially with both groups I/0.5 & I/3. Moreover, although that dermal collagen density in all groups was diminished significantly with a range between 23.5% and 38.1%, but collagen fibers in pTpT & liposomal with DNA repair enzymes treated rats appears healthy with no clumping or fragmentation, which was demonstrated by Masson's trichrome stain. Dilated dermal blood vessels and intravascular thrombosis with liposomal DNA repair enzymes similar dermal response to that of pTpT as in both group III/0.5, II/0.5, III/1.5, II/1.5 and III/3.

On the other hand, Yarosh et al (2001) confirm the ability of DNA repair enzymes in a liposomal vehicle when applied topically (T4 liposome lotion) for one year daily, to lower the rate of new skin cancers in patients with Xeroderma pigmentosum[17]. The findings of the current study revealed that the application of liposomal DNA repair enzymes showed similar results obtained with liposomal DNA repair

enzymes and pTpT with low dose irradiation 0.5 Gy. However, when 3 Gy was applied, PTpT were able to protect both the epidermis and dermis; while liposomal DNA repair enzymes could not defend the epidermis from the harmful effects of gamma irradiation.

In conclusion gamma irradiation is known to have harmful effects to the skin, which can be diminished by radioprotective agents. Thymidine dinucleotide (pTpT) and Liposomally Entrapped DNA Repair Enzymes are new photo-protective agents against ultraviolet through induction of DNA repair. When they were applied topically to rat skin to protect against ionizing radiation an obvious finding were noticed with gamma irradiation such as preservation of epidermis, minimal inflammatory cells and also it maintained the normal architecture of collagen bundles. However, pTpT gave a better response than Liposomally Entrapped DNA Repair Enzymes as radioprotectors against gamma irradiation. The current study assessed only the acute radiation effects which usually occur within the first four days of irradiation; further studies needed to evaluate the latent effects of gamma radiation on rat skin (weeks to months).

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