Skin Radioprotector (Diethone) Modifying Dermal Response of Radiation on Rats

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Investigations were carried out into the time-and dose-related changes in acute skin reaction following graded single dose (20, 30 and 40 Gy) of x-ray irradiation in Wistar rats, in order to evaluate the radioprotective effect of Diethon on skin. For the duration of skin response over 1. 5 score in dose of 40 Gy, the Diethone group of 24.7 days was significantly different ($p<0.02$) from that of control (29.8 days) and vaseline (29.2 days) groups, it was 17.1% diminution of skin response period compared with that of control group. By the averaging daily scores for 10 days during peak skin reaction the mean scores were obtained. Mean score of Diethone group (2.43 ± 0.22) was significantly different ($p<0.01$) from that of control (2.91 ± 0.23) and vaseline ($2.81\pm0. 18$) groups of 40 Gy dose. By iso-effect dose obtained at level of 2.5 score the dose reduction factor (DRF) was 1.41 which reduced radiation dose of 41% by radioprotective effect of Diethone.

From this experimental data, it may be possible to give higer radiation dose to large and/or radioresistant tumor mass rather than conventional treatment doses for improving therapeutic ratio by using topical application of skin radioprotector.

Key Words: Diethone, Skin Reaction, X-ray, Radioprotective effect

INTRODUCTION

After exposure of the skin to relatively high doses of ionizing radiation, an acute skin reaction develops over the next few weeks characterized by erythema, epilation and dry or moist desquamation with or without erosions. It affects the completion of treatment course of patient. These early acute changes are dose-dependent and reflect damage to the germinative cells of the epidermis and to the cutaneous vasculature.

A variety of animals have been used to assess the normal tissue damage to superficial structures. Field[1] showed that ealy skin reactions on rat feet were a reliable indicator of deformity developing within 6 months and this was confirmed by Brown and Probert[2], and other reporters[3,4]. A regenerative phase of cell replacement, characterized by sharply increased cell replication rates, occurs from the 3rd to 5th posstirradiation weeks. The postregenerative phase of hyperplasia reflects a temporary overshoot of cell density above control levels; a subsequent decrease in hyperplasia indicates feedback control of cellular proliferation[5].

The present study was performed to investigate the radioprotective effect of the Diethone by graded-single dose of radiation using leg skin of rats. For this purpose, the data analysis were made of time course, duration and mean score of skin reactions after irradiation. Finally, dose reduction factor (DRF) was obtained by iso-effect dose in dose-response curves.

MATERIALS AND METHODS

1. Experimental Animals

Male Wistar rats at 10~12 weeks of age were used for the experiments. They were housed two per cage in an air-conditioned room at a mean temperature of 22℃ and 50% of relative humidity with controlled light and dark cycles and were given free access to pasteurized pellets and water.

About 24 hours before irradiation, the animals were shaved from the hind legs with electrical clipper over an area approximately 3~4 cm in diamter.

2. Irradiation Technique

Irradiation were performed on unanesthetized

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rats contained in perspex holders with the leg to be irradiated being held away from the body by pegs. Four rats were mounted on a perspex tubes and were then covered by 3 mm thick lead plate in order to avoid any whole body irradiation by leakage or scatter irradiation, leaving an exposed field. To immobilize the animals, sets of prespex cages for each four rats were built. 12×12 cm^2 field size, that accomodated four rats at once, was used for each radiation modality with central shielding of foot in 6×6 cm^2 field size. The dose at the position of leg was measured using an Ionizing chamber (Victoreen Ltd.) with the center of chamber coinciding with the center of the leg. The x-ray was 140 KV and 15 mA using 2.0 Al filter in FSD 40 cm, the dose rate was 1.1 Gy/min. There was a variation of dose along the length of the field, but this variation was kept to a minimum by the putting the limbs as close as possible to the center of the long axis. The variation in dose for any group of four animals never exceed 5% of the mean value.

3. Administration of Drug

For the comparing of skin reaction with control group the vaseline was applied onto skin in the zone of irradiation prior to and after irradiation, and then in every morning and evening during course of experiments.

For the preventive purpose of skin reaction a 5 % of preparation (Diethone) was applied onto skin in the zone of planned irradiation 30 minutes prior to the irradiation.

After irradiation the skin is again greased with Diethone with intervals of one hour, and the skin was greased every morning and evening over the entire course of experiments.

Table 1. Study of Skin Reactions Using Different Radiation Doses and Drugs to the Hind Legs of Rats

Group	Dose (Gy)	No. of Animals	No. of Sites
Control ; (Radiation only)	30	4	8
	30	4	8
	40	4	8
Radiation + Vaseline	20	4	8
	30	4	8
	40	4	8
Radiation + Diethone	20	4	8
	30	4	8
	40	4	8

4. Assessment of Early Skin Reaction

An arbitrary scale of reaction level in this study was used similar to grading system for acute skin reaction modified from Fowler[11]. The skin reactions were recorded every day upto 42 days postirradiation using as arbitrary scale, and took a picture of left leg of all rats on every Monday.

RESULTS

1. Time Course of Skin Reaction After Irradiation

The time course of skin reactions in each groups given single radiation dose and drug application are shown in Fig. 1, 2 and 3. An early radiation damage in skin such as reddening appeared five to seven days after irradiation and reached a peak value (e.g. moist desquamation) between days 15 and 20, rose at the different rate in all the dose groups.

Table 2. Duration of Skin Reaction Over 1.5 Score

Dose (Gy)	Group	Duration of Response (Mean ± S.D.)	Diminution of Response (% of the Control
20	Control	11.7 ± 1.5	–
	Vaseline	12.3 ± 6.7	N.S.
	Diethone	12.7 ± 3.3	N.S.
30	Control	25.2 ± 3.9	–
	Vaseline	25.1 ± 3.9	N.S.
	Diethone	21.3 ± 3.3	15.4*
40	Control	29.8 ± 3.5	–
	Vaseline	29.2 ±2.1	N.S.
	Diethone	24.7 ± 3.6	17.1**

N.S. No significance

* the duration of skin reaction in Diethone group was singificantly different ($p < 0.1$) from that of control and vaseline groups.

** the duration of skin reaction in Diethone group was singificantly different ($p < 0.02$) from that of control and vaseline groups.

* The materials used in this work represents 2, 6-dimethyl-3, 5-diethoxycarbonyl-1, 4-dihydropyrimidine synthetized at the Institute of Organic Chemistry, Latvian SSR Academy of Sciences

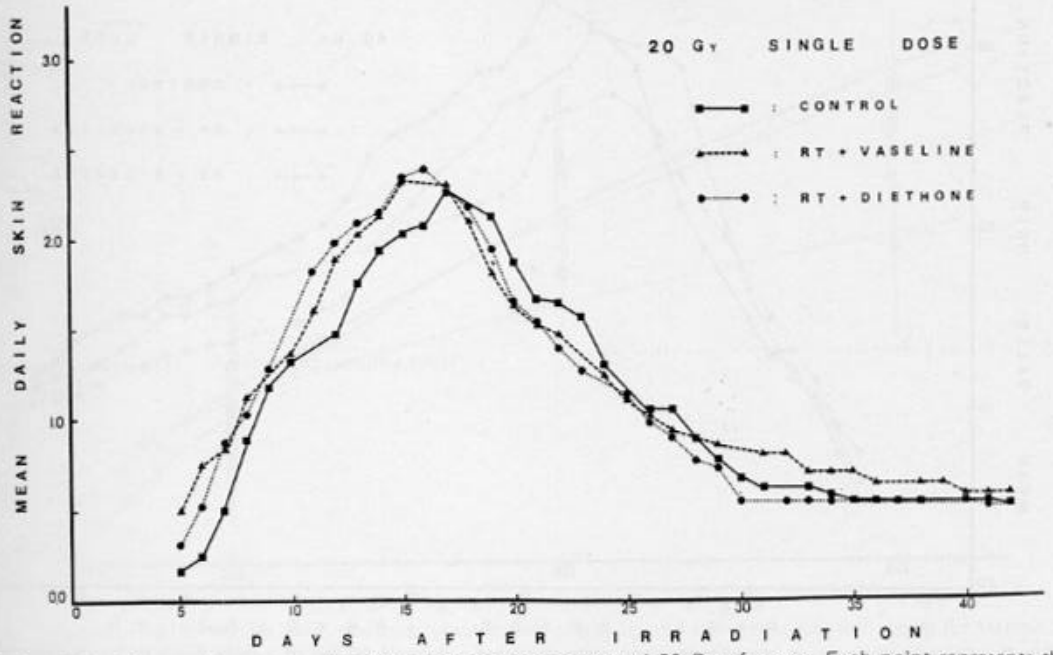

Fig. 1. Daily skin reaction scores with time after receiving a dose of 20 Gy of x-rays. Each point represents the mean of four animals, but the SEM for all points is not plotted for clarity of presentation.

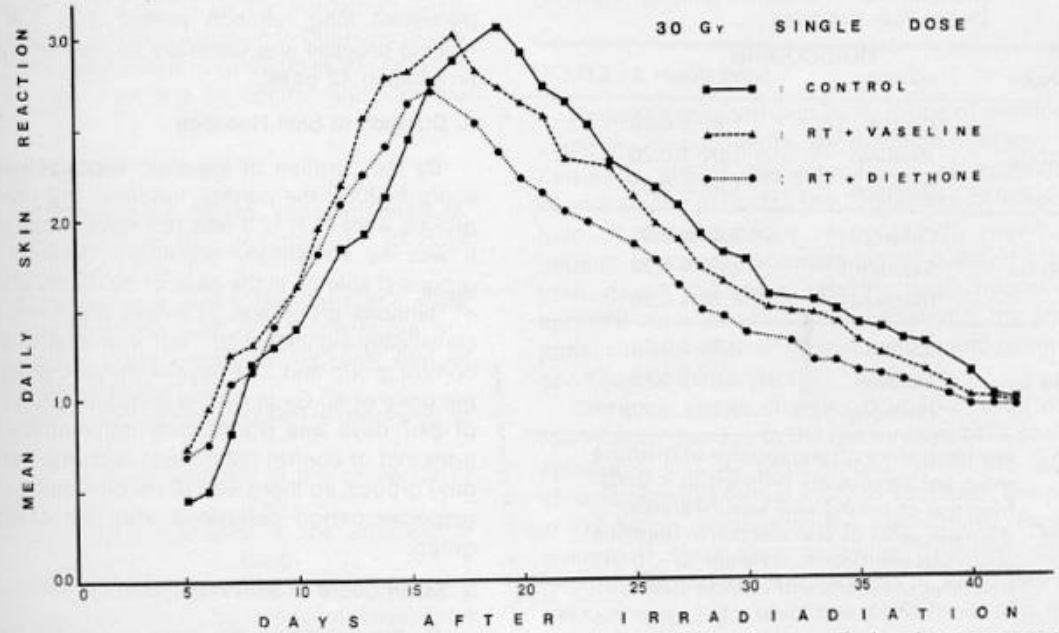

Fig. 2. Daily skin reaction scores with time after receiving a dose of 30 Gy of x-rays. Each point represents the mean of four animals, but the SEM for all points is not plotted for clarity of presentation.

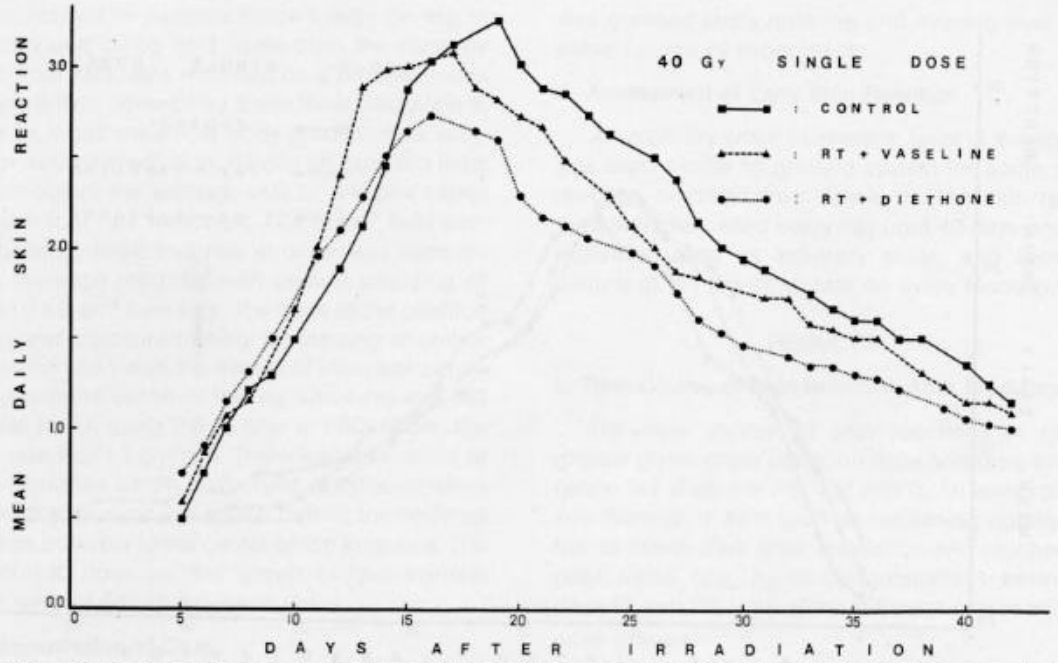

Fig. 3. Daily skin reaction scores with time after receiving a dose of 40 Gy of x-rays. Each point represents the mean of four animals, but the SEM for all points is not plotted for clarity of presentation.

Table 3. Mean Scores of Skin Reaction Obtained by Averaging the Daily Scores of 10 Days During Peak Reactions

Dose	Group	Score (Mean ± S.D.)
20 Gy	Control	1.91 ± 0.20
	Vaseline	1.97 ± 0.26
	Diethone	1.95 ± 0.33
30 Gy	Control	2.66 ± 0.36
	Vaselnie	2.68 ± 0.22
	Dientohe	2.36 ± 0.20*
40 Gy	Control	2.91 ± 0.23
	Daseline	2.81 ± 0.18
	Diethone	2.43 ± 0.228**

* the mean score of skin reaction in Diethone group was significantly different ($p < 0.05$) from that of control and vaseline groups.

** the mean score of skin reaction in Diethone group was significantly different ($p < 0.01$) from that of control and vaseline groups.

The skin reaction decreased sooner after the peak for 20 Gy dose of irradiation (Fig. 1), but the groups of 30 and 40 Gy dose revealed 1~2 days later and severe moist desquamation of skin with persistent long reaction period (Fig. 2,3). The healing process was generally completed by post-irradiation 42 days.

2. Duration of Skin Reaction

By the duration of the skin response over 1.5 score in 20Gy the control, vaseline, and Diethone groups were 11.7, 12.3 and 12.7 days, respectively. It was no statistically significant between each groups (Table 2). In the case of 30 Gy the duration of Diethone group was 21.3 days which reavealed statistically significant ($p<0.1$) from that of 25.2 in control group and 25.1 days in vaseline group. For the dose of 40 Gy in Diethone group, the duration of 24.7 days was significantly different ($p<0.02$) from that of control (29.8 days) and vaseline (29.2 day) groups, so there was 17.1% diminution of skin response period compared with that of control group.

3. Mean Score of Skin Reactions

By the averaging the daily scores for 10 days during peak skin reaction the mean scores were obtained ((Table 3). The mean scores of control,

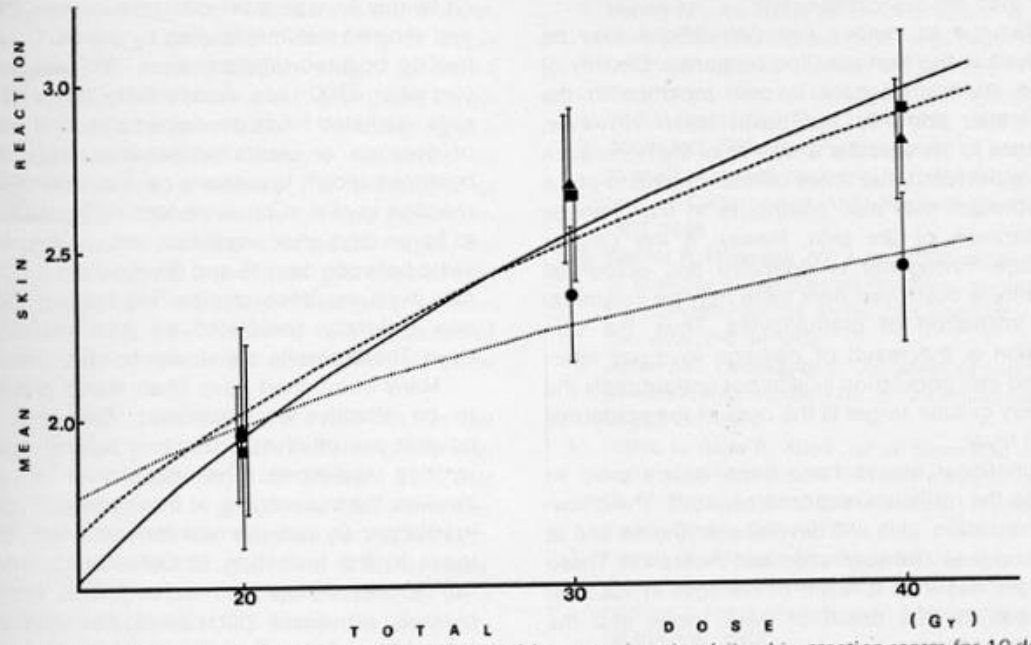

Fig. 4. Dose-response curves for skin reactions obtained by averaging the daily skin reaction scores for 10 days during peak reactions by single dose of x-ray in control (——), vaseline (– – –), and Diethone (------) groups.

vaseline, Diethone group were 1.91, 1.97 and 1.95, respectively by using of 20 Gy irradiation dose. The mean score of skin reaction in Diethone group were significantly different ($p<0.05$ in 30 Gy, $p<0.01$ in 40 Gy) from that of control and vaseline groups.

4. Dose-Response Curves

The dose-response curves were obtained by plotting the average skin reaction for 10 days during peak reactions (Fig. 4).

By dose-response curve the iso-effect dose were obtained at the level of 2.5 score, the iso-efect dose of control was 29 Gy, and that of Diethone group was 41 Gy.

The dose reduction factor(DRF) is descrived as follows

$$DRF = \frac{\text{dose of radiation in the presence of drug}}{\text{dose of radiation in the absence of drug}}$$

Therefore, DRF was $41/29=1.41$ which reduced the radiation dose of 41% by radioprotective effect of Diethone.

But there was some problem to fit a curve the data point shown in Fig. 4, because of statistical techniquees such as least squares regression or arbitrary cardinal scales for skin reactions.

DISCUSSION

Radiation-induced skin injury is one of the most common complications in radiation therapy, it frequently affects the completion of treatment course of the patients. The characters of magnitude of the cutaneous responses to radiation depend on several factors:radiation quality[1,6,7] the total dose[8] the dose rate[9,10], dose fractionation[11~13] the area or volume irradiated[14], the anatomic site and strains[15,16], and the age and general condition of the subject[17].

The early, acute changes probably result primarily from damage to the germinative cells of the epidermis and its appendages. while the later chronic changes follow injury to structures deeper in the dermis, particulatly the vasculaturez. Proliferation of these cells following treatment may restore the outward appearance of the tissue to near normal. Late reactions of fibrosis and necrosis have often been attributed to damage to connective tissues which, because of their slow proliferation, would not be manifested until much

later than the early response[3].

Damage to various cell populations may be involved in the skin reaction response. Clearity of prime importance here is cell depletion in the epidermis, primarily the basal layer. However, changes to the vascular elements of the dermis are also important. Later times cellular depletion of the endothelium may also contribute to the changes appearance of the skin. Finally, if the cellular damage throughout is extensive and epidermal integrity is destroyed then there may be inflammatory infiltration of granulocytes. Thus, the skin reaction is the result of damage to many interrelated cell population in skin but undoubtedly the primary cellular target is the cells of the epidermal basal layer.

Functional assays have been widely used to assess the radiation response of skin[18~22]. Following irradiation, skin will develope erythema and at higher doses. desquamation and ulceration. These changes occur as a result of changes in vascular permeability, the death of basal cells, and the inability of the survivors to replace the outer layers of cells, which are lost due to normal "wear and tear" processes[23].

Skin erythema after X-irradiation is typically characerized by a transient reddening of the skin in human, but not generally in rodents, within a few hours of exposure. This fades and is replaced by a more prolonged highly dose-dependent reddening, the third phase beginning-5 weeks after irradiation. The second phase reaction generally starts after 5 to 7 days and the intensity may increase over the next 12 weeks until a peak is reached after which regenerative healing responses overtake the cell depletion processes. If howerver, the dose was sufficiently severe the regenerative healing processes may not be able to compensate for the damage and the integrity of the skin structure will be broken[24].

Numerical scoring systems have been set up to assess radiation damage, and these systems can be used to generate dose-response curves[12,16,19]. Although the changes seen in animal skin following radiation differ somewhat from those occurring in human skin, primarily because of anatomical differences, the results from animal studies have yield valuable information on certain aspects of the skin' s response to radiation. Because of swine skin has some anatomical similarity to human skin, it has been used to study the gross and microscopic changes following irradiation[25]. After single exposures of 1700~2700 rads a moist reaction appeared by day 17, was most extensive on days 24~28, and showed maximal healing by day 36. Complete healing occured regularly after 1700 rads but was rare after 2700 rads. Above dose levels of 2300 rads irradiated fields developed a second reaction of necrosis or ulceration between days 36~70 postirradiation[5]. In author's case an ealy radiation reaction in skin such as reddening appeared five to seven days after irradiation and reached a peak value between days 15 and 20, rose at the different rate in all the dose groups. The healing process was generally completed by postirradiation 42 days. Those results are similar to other reports[5].

Many compound have been tested and found to be effective as protectors. Sulfhydryl compounds are efficient protectors against sparsely ionizing radiations. The mechanism of action involves the scavenging of free radicals[26]. During irradiation, O_2-radicals are formed which in turn leads to the formation of OH-radicals. The O_2-radical has in itself no or very little effect on biologic processes but participates thus in the formation of OH-radical. Among the factors conditioning a high radiosensitivity and radioaffection of skin and other tissues, there should be mentioned the development of lipoperoxidation processes in cellular membranes and the development of free radical processes.

Ohlsen et al[27] experienced that in a sereis of experiments in rabbits the dermal reaction, provoked by a single dose or intermittent doses of irradiation, was prevented or modified by topical or parenteral administration of local anesthetics, compared to irradiated control animals. The topical application of a eutectic lidocaine/prilocaine cream, ELMA 5% was found to be more effective than intravenously injected lidocaine (Xylocaine 1 %), but it was a pilct study and the results should be further investigated for clinical application.

The new drug developed by the Institute of Organic Chemistry, Lativian SSR Academy of Sciences, is used in medical practice in the form of 5 % cream of Diethone on vaseline base. Its radioprotective action is conditioned by such physicochemical properties of Diethone as capacity to prevent peroxidation of lipids and stabilize cellular membranes as well as to absorb free radicals[28].

Diethone is a local-action preparation it has a clearly pronounced antioxidant activity, normalizes oxidation processes in irradiated tissues, hinders the development of processes of peroxy-type oxidation of lipids in cellular membrane and their disintegration, prevents the development of strus-

tural change in the cells of epidermis and subcellular structures, lowers the permebility of capillaries, exhibits a detoxicating effect, facilitates normalization of the tissue metabolism, and stimulates regeneration processes[28]. At covering the skin before irradiation Diethone increases its radioresistance or decreases the symptoms of radiation dermatitis: erythema, dry and moist desquamation. In the case of radiation dermatitis, the drug removes edema, hyperemia, pruritus and skin burning and accelerates its healing. The main mechanism of action is related to stablizaion of cellular membranes and absorption of free radecals[28]

The present experiments showed that in the case of 30 Gy the duration of Diethone group was 21.3 days which revealed quite different from that of 25.2 days of control group and that there was 17.1% diminution of skin response period in Diethone compared with that of control of 40 Gy dose. By dose-response curve the iso-effect dose were obtained at the level of 2.5 score, the iso-effect dose of control was 29 Gy and that of Diethone group was 41 Gy. Therefore, dose reduction factor (DRF) was 41/29=1.41 which reduced the radiation dose by radioprotective effect of Diethone.

It thus concluded that the ability of skin radioprotector to be pronounced antioxidant activity, to prevent peroxidation of lipids and stabilize cellular memranes as well as to absorb free radicals would result in an increased resistance to radiation injuries, therefore it may be possible to give higher radiation dose to large and/or radioresistant tumor mass rather than conventional treatment of radiotherapy by using preventive or therapeutic purpose of skin radioprotector.

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═ 국문초록 ═

방사선 보호제(Diethone)의 랫드 피부반응에 대한 수식작용

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Diethone의 피부에 대한 방사선 보호작용을 평가하기 위하여 Wistar 계 랫드에 단일 X-선(20, 30, 40 Gy) 조사후 시간경과와 선량변화에 따른 급성 피부반응을 연구하였다. 40 Gy 선량에서 Diethone 군의 피부반응기간은 24.7일로 대조군(29.8일)과 바셀린(29.2일) 투여군에 비하여 약 17%의 피부반응 기간이 뚜렷이 감소 되었다. 피부반응이 최고도에 달한 10일 동안의 평균치는 Diethone 군(2.43)이 대조군(2.91)과 바셀린(2.81) 군에 비하여 현저히 감소되었다. 피부반응 Score 2.5에서 구한 등가효과 선량의 반응감소 계수(DRF)는 1.41로 피부보호효과가 있었다.

본 실험결과로 보아 방사선 피부보호제를 국소적으로 도포함으로써 일반적인 치료선량보다 많은 방사선량을 거대하고 방사선내성이 있는 종양에 조사함으로로써 치료효과를 향상시킬 수 있을 것으로 기대한다.