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PROTECTION OF THE SKIN OF MICE AGAINST IRRADIATION WITH CYCLOTRON-ACCELERATED HELIUM IONS BY 2-MERCAPTOETHYLAMINE

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The availability of megavoltage radiation as a modality for cancer therapy has largely reduced the significance of skin tolerance as a limiting factor in treatment planning. There remain two circumstances in which the risk of complications from excessive radiation injuries to the skin may limit the use of this modality. These occur when the adjacent radiation fields must be matched and therefore risk overlap, and when the skin must be in the radiation field in order to re-treat for recurrent disease.

Currently, the use of high linear energy transfer (LET) radiation (ARCHAMBEAU et coll. 1974, BROWN et coll. 1973, WITHERS 1973) as a treatment modality is being actively explored. Due to the higher RBE associated with these radiation qualities, it seems likely that this would also be a situation in which the skin tolerance could become an important, perhaps limiting, consideration. Local protection of the skin would, under some circumstances, permit higher doses of radiation to be delivered to the tumor volume.

Among the first to demonstrate that a local protective effect could be obtained was FORSSBERG (1950) who found that a subcutaneous injection of 2-Mercaptoe-

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thylamine (MEA) in guinea pigs could prevent radiation induced epilation. DARCIS & GILSON (1967) and DARCIS & HATTERBECK (1958) irrigated the vaginal mucosa and rectum of mice with an MEA solution before irradiation and observed that the number of abnormal exfoliated cells produced by the radiation was significantly diminished, presumably due to the protective effect of MEA.

FOGH (1960) observed a significant degree of protection when a 1 per cent suspension of MEA in Vaseline was applied topically to a small area of rabbit skin two hours before irradiation. BACQ et coll. (1961) found that percutaneous injection of MEA would provide local protection of mouse skin from epilation. GOEPP et coll. (1967 a, b, 1968, 1969), have demonstrated that a single drop of saturated aqueous solution of MEA applied to the tongue of a mouse before irradiation produced a significant protection (dose reduction factor of 1.35) to the mucosal surfaces. The possibility that a topical application of MEA would protect the oral mucosa of mice against repeated exposures to roentgen irradiation was also investigated by ANTONE & GIBBS (1973).

LOWY & BAKER (1972, 1973) reported that the topical application of a 10 percent suspension of MEA applied to the skin of mice 15 minutes before irradiation with 250 keV roentgen rays resulted in a dose reduction factor of 1.6, 1.4 and 1.2, using an acute dry desquamation, moist desquamation, and necrosis, respectively, as end points. The same authors observed that topical MEA also seemed to reduce some of the late sequelae of radiation injury to the skin.

In view of these data, investigations were undertaken to determine whether any local protection from radiation injury to the skin from high LET radiation by the topical application of MEA could be demonstrated.

Materials and Methods

Female mice (C_3H/HeJ, Jackson Laboratories, Bar Harbor, Me.) were used. The mice were housed five per cage and offered food (Simonsen Laboratories, Gilroy, Ca.) and water ad libitum. They were 9 to 10 weeks of age and 20 to 24 gram in weight at the time of irradiation.

For high LET radiation, the 184-inch cyclotron of the Lawrence Radiation Laboratory, Berkeley, California, was used. The cyclotron produces a beam of helium ions of 910 MeV initial energy. The quality of the radiation was modified as previously described (RAJU et coll. 1969, 1972) resulting in the depth dose distribution appearing in Fig. 1. An oval brass aperture 20 mm × 18 mm placed immediately in front of the mouse leg provided the collimation. The dose rate at the skin surface was 350 rad per minute.

The MEA for topical application was prepared as follows: 2 g of the drug (Cal Biochem, Los Angeles, Ca.) was mixed with 1 ml of distilled water and a sufficient volume of saturated sodium bicarbonate solution to bring the pH to 6.5 to 7.5. Approximately 1 ml was required. Unibase (Parke, Davis & Co., Detroit, Mich.)

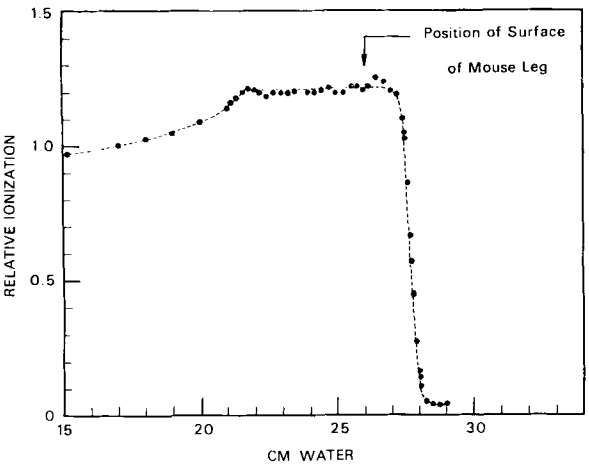

Fig. 1. Schematic of modified Bragg curve used in irradiation of mouse legs. The position of the surface of the leg for the extended Bragg peak experiments is indicated in the figure. The points indicate actual ionization chamber readings and a smooth curve has been drawn through the points.

was added to bring the drug concentration to 10 per cent by weight. The mixture was blended to a smooth paste. A placebo for the control animals was prepared in which the MEA was omitted. Fifteen minutes before irradiation, the right legs were covered by either the protective or nonprotective (placebo) creams.

Table 1

Grading system for skin irradiation reactions

Tissue grades and appearance in acute injury phase	Tissue grades and appearance in recovery phase
1.0 no difference from controls	1.0 no difference from controls
1.5 slight erythema	1.5 limb appears normal except for presence of hair depigmentation
2.0 distinct erythema	2.0 hair is depigmented and there is sparse regrowth of hair
2.5 suggestion of dry desquamation	2.5 sparse regrowth of hair; probably no edema; the skin does not appear as thin as in a 3.0 recovery state
3.0 dry desquamation, powdery appearance of skin on close observation with small cracks and flaking of skin; usually edematous	3.0 marked epilation, skin is thin appearing and presents a tight appearance; may be a reduction in the size of the limb
3.5 dry desquamation with suggestion of incipient skin breakdown	3.5 very thin, shiny skin, usually edematous
4.0 moist desquamation of moderate extent, patchy appearance	4.0 focal areas of moist desquamation with extensive scab formation
4.5 major percent of limb is involved in moist desquamation, may find small areas of necrosis	4.5 small, nonhealing areas
5.0 significant amount of necrosis with loss of dermis, similar to a third degree burn	5.0 open, but nondraining wound

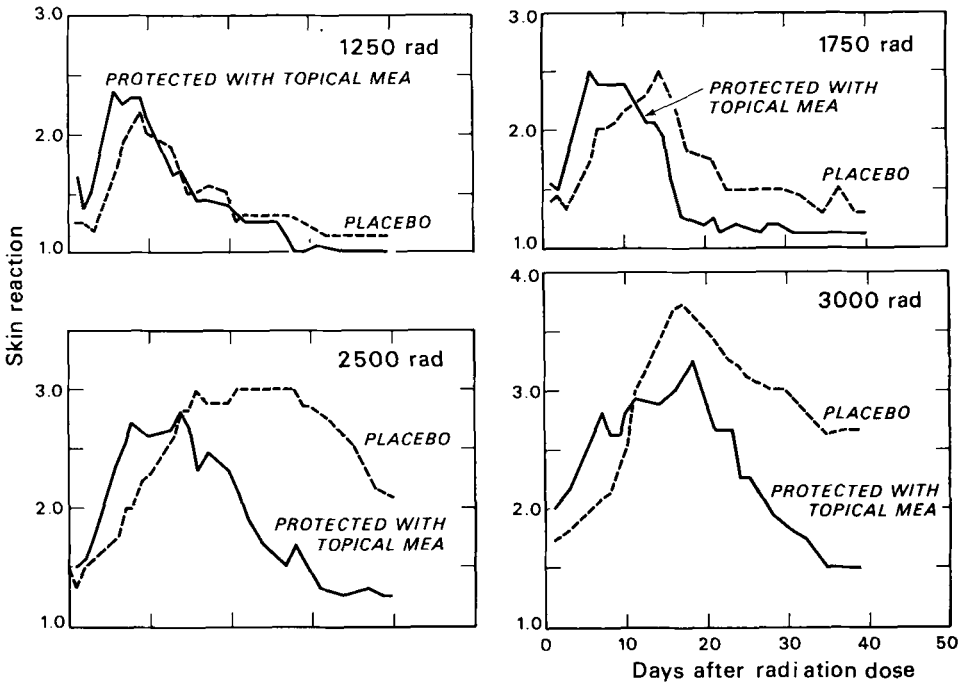

Fig. 2. Development of acute skin reactions after different doses of cyclotron-accelerated helium ions.

One day before irradiation, the hair on the right leg was removed using a depilatory agent (LOWY & BAKER 1973). For irradiation, the animals were anesthesized with sodium Pentobarbital injected intraperitoneally (35 mg/kg) and the leg placed in the center of the radiation beam as previously described (RAJU et coll. 1969, LEITH et coll. 1974).

The acute skin reactions were observed daily and graded by a numerical system (Table 1) to which the skin reactions could be related.

Results

The dose response curves for the protected and for the unprotected skin reactions of helium ion irradiated mouse leg are shown in Fig. 2. These two irradiation conditions were compared by using the areas underneath the skin reaction curves (days one to 30 after irradiation) as an index of the dose-dependent, total skin reactions. The area underneath the curve divided by the number of days gives the average skin reaction per day (FOWLER 1967, DENEKAMP et coll. 1966, HEGAZY & FOWLER 1973). Tables 2 and 3 list average skin reactions for the MEA-protected and unprotected mice. In Fig. 3, these values have been used to obtain dose-modifying

Table 2

Values for average early (one to 30 days postirradiation) skin reactions in unprotected and MEA protected mice

Dose level (rad)	Condition	
	MEA protected	Unprotected
1 250	1.31*	1.36
	(8)**	(8)
1 750	1.42	1.67
	(8)	(8)
2 500	1.89	2.84
	(8)	(9)
3 000	2.54	3.30
	(7)	(4)

* indicates the mean value of the average early skin reaction.
** the number in parentheses indicates the number of animals from which the mean value of the average early skin reactions were obtained.

factors (DMF) for selected levels of injury response. For average one to 30-day postirradiation scores of 1.5, 2.0, and 2.5, the DMF values are:

$$DMF_{(1.5)} = \frac{1\,890 \text{ rad MEA}}{1\,530 \text{ rad placebo}} = 1.24$$

$$DMF_{(2.0)} = \frac{2\,590 \text{ rad MEA}}{2\,070 \text{ rad placebo}} = 1.25$$

$$DMF_{(2.5)} = \frac{2\,960 \text{ rad MEA}}{2\,370 \text{ rad placebo}} = 1.25$$

Although the dose response curves in Figs 2 and 3 give indications of the protection afforded by topically applied MEA, it has been suggested by KELLERER & BRENOT (1973) that difficulties can arise in derivation of modifying factors such as DMF from uncertainties in interpolation and curve-fitting of dose effect relations, particularly since evaluation of skin reactions is based on a numerical grading system having an inherent subjectivity. Effects measured using an ordinal scale may therefore be analyzed using nonparametric statistics, such as the Mann-Whitney rank order test (SIEGEL 1956). It is suggested that this test will allow estimation of the skin responses after helium ion irradiation with MEA protection which are most similar to the helium ion skin reactions after different doses without chemoprotection. In Table 4, this test has been used to compare mice protected either with topical MEA or placebo. This allows establishment of the dose comparisons which are not otherwise statistically acceptable as being equivalent. If such dose comparisons are not valid, the ratio of doses (and therefore DMF) has no relevance. In these comparisons the 5 per cent level of significance for discrediting the null hypothesis was chosen.

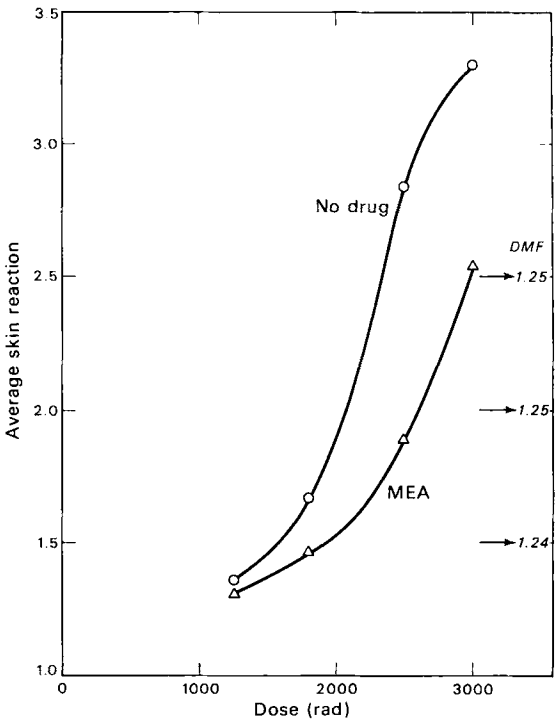

Fig. 3. Dose response curves of the average one- to 30-day post irradiation skin reactions for mice irradiated with single doses of modified Bragg peak helium ions, either unprotected (placebo) or protected by topical application of MEA. The DMF for various levels of effect is indicated.

Discussion

The data indicate that a dose-modifying factor of approximately 1.25 may be obtained in single dose exposures to cyclotron-accelerated helium ions by the use of topically applied MEA. The skin reactions of the protected animals all show an early peak in the gradings at 7 to 9 days, several days before the development of the skin reactions in the non-protected animals. This is a transient response due to the

Table 3

Values of maximum skin reaction and time after irradiation when maximum reaction is first attained

Dose level (rad)	MEA protected		Unprotected	
	Maximum skin reaction	First day of maximum skin reaction	Maximum skin reaction	First day of maximum skin reaction
1 250	2.3	6	2.2	9
1 750	2.5	6	2.5	14
2 500	2.8	14	3.0	16
3 000	3.2	18	3.7	17

Table 4

Comparisons of skin reactions over the period of one to 30 days postirradiation

	Dose (rad)				Range of unacceptable DMF	
	3 000 control	2 500 control	1 750 control	1 250 control		
1 250 MEA	*	*	*	NS	0.7	—
1 750 MEA	*	*	NS	NS	0.7	—
2 500 MEA	*	*	NS	*	1.0	2.0
3 000 MEA	*	NS	*	*	1.0	1.7

An asterisk indicates that comparison of the total skin reactions over the period of one to 30 days postirradiation are significantly different at the 5 per cent level of confidence using the Mann-Whitney rank-order test. As a corollary, the ratio of doses of MEA protected/placebo protected mice is excluded from the limits of acceptable DMF values.
NS indicates a comparison of dose effect responses which are not statistically different at the 5 per cent level of confidence, and therefore indicate an acceptable DMF comparison.

irritating action of the MEA or depilatory agent. A similar effect may be produced by plucking the hair immediately before irradiation (HEGAZY et coll. 1973). Such irritation decreases the cell cycle time and skin reactions appear somewhat earlier than in nonstimulated skin. One result of the irritating action of the chemical agents is to increase the area under the 30-day dose–response curve and therefore effectively decrease the DMF estimate. The real protection afforded by the topical MEA is therefore probably 15 per cent greater than indicated by the DMF of 1.25.

Because of the extension of the Bragg curve by interposition of a ridge filter, the modified Bragg curve (Fig. 1) represents a family of curves of varying energy and, consequently, varying LET. Therefore, the LET at the surface of the skin may not be expressed as a single LET value, but rather should be expressed as dose per unit interval of LET spectrum. The LET distribution at the surface of the mouse skin is not currently available; however, the modal LET is probably approximately 15 keV/micron.

In their investigations of skin reactions of mice, YUHAS & STORER (1969) found a DMF of 2.4 for production of ulceration in 50 per cent of an irradiated skin field after systemic protection by intraperitoneal injection of WR 2721. In agreement with LOWY & BAKER (1973), a diminished severity of skin reaction was found in the present investigation and also a more rapid repair of the clinically demonstrable skin injury in the protected animals, even after irradiation with helium ions where the modal LET is increased by a factor of 7 over the 250 keV roentgen rays (JOHNS 1956).

It is assumed that the protective effect of MEA is asserted by influencing the

indirect mechanisms. If the spectrum of LET values of the accelerated helium ions is similar to the LET spectrum for neutrons described by ROSSI (1964), then the proportion of high energy events (expected to produce injury by direct action) would not be expected to change significantly with dose.

ROSSI, however, found that the LET spectrum for the γ-radiation of ^{60}Co (low LET) showed an increase in the frequency of high energy events (proportion of damage by direct action) as the dose increased. These observations may explain why LOWY & BAKER (1972, 1973) found a decrease in the protective effect of MEA as the roentgen-ray dose increased, while in the present investigation, with a higher modal LET value, a constant DMF over the range of doses used was found. It has been shown (LEITH et coll. 1974) that recovery between equal size fractions of helium ions, in the modified Bragg peak region of conization, split over a 24-hour period, is about 80 per cent of the comparable recovery occurring between equal-sized fractions of 230 keV roentgen rays.

It is interesting that a differential (cell cycle dependent) protective action of MEA for cell lethality in Chinese hamster cells has been demonstrated by SINCLAIR (1968, 1969). He found that cells in radiation sensitive stages (G_2 and M) of the cell cycle were most protected. Mouse skin has approximately 10 to 15 per cent of epidermal basal cells in the G_2 phase (GELFANT 1965), which may suggest that topical protection of skin by MEA may be achieved even with radiations of relatively high LET.

It is of interest to consider the possible beneficial effects of topically applied MEA with regard to a tumor response after heavy particle radiation. For example, the data of ROCKWELL & KALLMAN (1973) show that, at a single roentgen-ray dose of 2 500 rad, which in our helium-ion irradiated mice causes a maximum skin reaction just below the threshold for moist desquamation (Fig. 2), approximately 30 per cent of EMT6 solid sarcoma tumors will be cured. If the skin is protected (DMF = 1.25), this means that a biologically equivalent dose of 3 125 rad to the skin could be given. If this were done, it should increase the percentage of cures of the solid tumor to about 65 per cent. The RBE of these accelerated helium ions in the modified Bragg peak region relative to 230 keV roentgen rays is about 1.2 to 1.3 (LEITH et coll. 1974, RAJU et coll. 1971, 1972). Therefore, in the care of the tumor this increase in RBE would further increase the therapeutic ratio.

It seems reasonable to suggest that topically applied MEA may be clinically useful to obtain a skin sparing effect in those circumstances in which excessive irradiation of the skin is unavoidable. The clinical implications of the comparison deserve further consideration.

Acknowledgements

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SUMMARY

Mouse skin was exposed to doses of 1 250 to 3000 rad using a helium ion beam with modal LET of 15 keV per micron. The skin reactions were evaluated for mice treated with a topical application of 10 per cent MEA in a cream base or a placebo 15 minutes before irradiation. A comparison of the skin reactions indicated that the MEA treatment resulted in a DMF of at least 1.2. The implication for radiation therapy was discussed.

ZUSAMMENFASSUNG

Mäusehaut wurde mit Dosen von 1250 bis 3000 rad unter Verwendung eines Helium Ion Strahls mit einer modalen LET von 15 keV per Mikron bestrahlt. Die Haut-Reaktionen wurden bei Mäusen, die mit einer lokalen Applikation von 10% MEA in einer Krem-Base oder einem Placebo 15 Minuten vor der Bestrahlung behandelt worden waren, festgestellt. Ein Vergleich der Hautreaktionen deutet darauf hin, dass die MEA Behandlung zu einer DMF von mindestens 1,2 führt. Die Bedeutung für die Strahlentherapie wird diskutiert.

RÉSUMÉ

La peau des souris est exposée à des doses entre 1 250 et 3 000 rad au moyen d'un faisceau d'ions d'hélium avec un modal de LET de 15 keV par micron. La réaction de la peau est mesurée dans les souris traitées avec une application locale de crème de MEA à 10% et avec un placebo, 15 minutes avant l'irradiation. Après comparaison des réactions de la peau, on remarque que le traitement par MEA résulte en un DMF d'au moins 1,2. On en discute l'implication en radiothérapie.

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