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REDUCTION OF RADIATION-INDUCED HAIR LOSS BY TOPICAL APPLICATION OF RADIOPROTECTORS

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ABSTRACT

Our transdermal permeation studies of radioprotectors in permeation-enhancing vehicles led us to hypothesize that radiation-induced hair loss could be reduced by topical application of radioprotectors. In the present study we used a hair regrowth assay in "plucked" mice to measure the radioprotective effectiveness of WR-1065, cysteine and TEMPOL when they were dissolved in a variety of vehicles, i.e., saline, dimethyl formamide (DMF), propylene glycol (PG), dimethyl sulfoxide (DMSO), ethanol. Protector effectiveness varied with radiation dose and vehicles. At 600 R, WR-1065 or cysteine, in any vehicle, reduced radiation-induced baldness scores; at 800 R, only WR-1065 in DMF and cysteine in saline, produced some protection. TEMPOL in ethanol produced protection at both the 600 and 800 R. Results suggest that suitably chosen topical application of protector/vehicle combinations can reduce radiation-induced hair loss.

INTRODUCTION

Hair loss in cancer patients is a troublesome side effect of ionizing radiation and chemotherapy. Attempts at reducing baldness using tourniquets and ice packs have been ineffective (1-3). Protection against hair loss and other skin reactions has however

been afforded with systemic administration of radioprotectors in rodents (4-5). However, such **systemic** administration of protectors introduce complications such as toxicity and protection of the tumor itself which have so far precluded their use (6). Regarding hair loss, these two problems would become irrelevant if topical application of protectors were sufficiently effective. There have been a few studies concerning topical radioprotection, mostly utilizing water-based vehicles, but most have not been successful (4,7). We assume that lack of significant topical effectiveness is related to difficulty of the hydrophilic protectors to pass the outer, dead, hydrophobic layers of the skin to reach the deeper, living, radiation-sensitive targets.

Hoping to improve penetration of protectors to the radiosensitive targets in the skin, we previously investigated the passage of radiolabelled protectors (WR-2721, cysteine and prostaglandin E_2) through the skin of mice (8) and rats (9). We compared passage of each dissolved in water versus known permeation enhancing vehicles such as dimethyl formamide (DMF), dimethyl sulfoxide (DMSO), and propylene glycol (PG). For a protector to be effective it must traverse the non-living outer stratum corneum to reach the dividing cells of the avascular epidermis and deeper vascular dermis with its hair follicles. An additional consideration with the hydrophilic protector WR-2721 is that after transport across the hydrophobic stratum corneum barrier it must reach the endothelium of blood vessels (of the dermis) to be dephosphorylated to its active form, WR-1065 (10). Systemically delivered, WR-2721 (S-2-(3-aminopropylamino) ethylphosphorothioic acid) protects against acute (4) and chronic (5) radiation damage to skin and its hair follicles. Its failure to protect when applied topically is reasonably attributed to its inability to penetrate the outer layers of skin and/or its failure to be converted to WR-1065 (S2-(3-aminopropylamino) ethanethiol) in the mitotically active avascular epidermis.

The work reported here demonstrates that, when applied topically in suitable permeation-enhancing vehicles, WR-1065, cysteine and TEMPOL (11) can reduce radiation-induced hair loss.

MATERIALS AND METHODS

Animals

$C_{57}Bl$ female mice were purchased at 7 weeks of age (Jackson Laboratories, Bar Harbor, ME), housed 5

per cage, 12 h light/dark cycle at 72°F and fed Purina Lab Chow and water ad libitum. They were cared for in AALAC approved animal quarters under strict guidelines of the Animal Care Committee of Temple University.

Protocol

At 8 weeks of age mice were lightly anesthetized (Nembutal, 50 mg/kg) and the hairs of their lower back were plucked creating a 25-30 mm bald spot. Plucking initiates the synchronous anagen (growing) stage in hair follicles (12-13) during which the dividing cells of the hair matrix are highly sensitive to ionizing radiation (14). Interruption of growth and the subsequent regrowth rate can be quantified by measuring the time taken to regrow. About 14 days after plucking, when all hairs were in the anagen stage (as evidenced by emerging new black hairs), mice to be irradiated were anesthetized. The skin patch with emerging new hairs was degreased by gently washing with soap, rinsed and air-dried prior to application of various protector-vehicle combinations. A cotton pleget inside a 2.5 cm semi-rigid polypropylene Hilltop chamber (Hilltop Research, Cincinnati, OH) was saturated (0.2 ml) with the protector-vehicle solution. The chamber was affixed to the area of skin to be irradiated with transparent tape and left for a period of 1 hour, a time sufficient for protector penetration based on our earlier radiolabelled studies in the mouse (8).

Radiation

At the end of the 1 hour application period, mice were placed under a 2 mm lead shield with a 20 mm hole directly over the Hilltop chamber so as to irradiate only the protector-treated area of regrowing hairs. The whole body dose under the lead shield was calculated to be less than 10% of the unshielded dose. The animals received 400, 600 or 800 R (measured with a Victoreen R-meter) from a G.E. Maxitron 300, operating at 300 kVp, 20 mA, STD 50 cm, with 0.5 mm Cu filtration. This range of doses was chosen since in unprotected controls receiving 100 -1600 R it resulted in baldness scores ranging from 1.0 (full hair coat) to 4.0 (completely bald; Figure 1). After irradiation, chambers and protectors were removed and the treated skin was again gently washed and air-dried.

Hair Loss Assay

Based on our preliminary studies, depending on dose, hair begins to fall out about 5 days post-radiation; by 7-10 days baldness occurs. Regrowth of the hair was evaluated by a modification of other reported scoring systems (11, 15-19). It was independently scored by 2 observers, approximately every other day, until 30 days post-radiation. The baldness scoring system was as follows: 1 = normal hair density (100%); 2 = 50% hair; 3 = 25% hair; 4 = bald (0%). The scores from both observers were averaged. This system is very similar to that reported by others (11,15-19).

Protectors and Permeation Enhancers

Based on their solubilities, protectors were dissolved in their most efficient permeation-enhancing vehicles (8).

WR-1065 (Developmental Therapeutics Program, Division of Cancer Treatment, NCI) was dissolved (a) in saline alone, (b) in saline and mixed 1:1 with propylene glycol (PG) and (c) in a 7:3 mixture of saline and dimethyl formamide (DMF). Final concentration of WR-1065 was 50 mg/ml in all cases.

Cysteine (hydrochloride) (Fisher Scientific) was dissolved in (a) in saline alone and (b and c) as a 1: 1 mixture of cysteine in saline and PG or dimethyl sulfoxide (DMSO). Final concentration of cysteine was 100 mg/ml.

TEMPOL (Aldrich Chemical) was dissolved in 70% ethanol to a concentration of 70 mg/ml (11).

Control animals

These animals received no protector - permeation enhancer combination treatments other than saline. Mice received 100 -1600 R X-radiation to measure the full dose-response of the hair regrowth assay.

Data Analysis

The mean baldness score and the SEM for each group of 4-11 mice on each observational day was plotted versus time. With doses below 800 R in control and experimental groups, the observed peak baldness scores ranged from 1 (no detectable hair loss) to 4 (total baldness). For groups receiving more than 800 R, the

assay system appeared saturated, i.e., irradiated areas were nearly all bald (scores near the maximum of 4.0 regardless of dose). However, in groups receiving 800-1600 R recovery from total or near total hair loss was progressively delayed. This suggested that higher doses inflicted progressively greater radiation damage to the cells in the hair follicle thus delaying recovery. This leads us to conclude that our actual injury score would have exceeded the maximal observation baldness score of 4.0 if the method allowed. Since it did not, we feel justified and compelled to estimate the theoretical maximal damage. This we did by determining an "extrapolated" peak by fitting linear lines that best corresponded to the intersection of the ascending and descending slopes of the measurable baldness response curve against time. The slopes and Y-intercepts of these lines were calculated by linear regression analysis. Thus derived, the magnitude of the "extrapolated" peak value (a measure of follicular damage) and day of the "extrapolated" peak value were estimated from the graphs. Modification factors (MFs) based upon extrapolated or observed peaks were calculated by dividing the values from saline controls by those of experimentals receiving protector-vehicle combinations. Similar calculations of MFs were made for time (days) to attain full hair regrowth. Since the 600 R controls had almost full regrowth by day 30, the MFs were calculated with the assumption that control regrowth had occurred on day 30. MFs greater than 1 indicated that either peak damage was not as severe or that hair regrowth occurred sooner in the treated groups i.e., protection occurred. The MFs for the day of the peaks were obtained by dividing the day of peak values from the treated groups by days from the control groups. MFs greater than 1 indicated a delay in the onset of damage which we interpreted as protection.

Peak baldness scores of treated animals exposed to 600 R were compared to peak baldness scores of control animals using the Student's t-test. P values less than 0.05 were considered to be statistically significant.

To determine whether additional protection occurred when the protector was dissolved in permeation-enhancing vehicles, the MFs from these animals were compared to those obtained from animals treated with the protector in saline. These results were expressed as a percent increase and are listed in Table I.

RESULTS

Controls

Figure 1 demonstrates baldness scores in unprotected control animals receiving 100-1600 R. Data obtained from animals receiving 100, 200 or 300 R were combined since they all had identical scores (1.0) throughout the observational period. Similarly, data from animals receiving 800 or 900 R were combined. To avoid confusion, the SEMs were not plotted but the ranges are indicated in the accompanying figure legends.

Peak baldness scores generally occurred within the first 10 days post-irradiation. This peak was dose dependent between 100 R and 900 R; all doses above 900 R produced a score of 4.0, i.e., total baldness. With respect to the time for full or partial recovery, there appeared to be a dose dependent response with most doses of 900 R or less producing full regrowth of hair within 30 days; at and above 1200 R only partial recovery was seen by 30 days.

Baldness scores and extrapolated peaks are presented in graph form (Figs 2 & 3) for only two of the treatment groups as they are representative of the graphs obtained from the other treatments. The calculated MFs for all treatment groups are presented in Figs 4 & 5). All animals given WR-1065, cysteine or TEMPOL and exposed to 400 R had baldness scores identical to controls, i.e. there was no radiosensitization. These data are not presented below.

WR-1065/Saline (Fig 2)

600 R: Prior to day 10 animals receiving WR-1065 had an observed peak baldness score significantly less ($P < 0.005$) than that of saline controls indicating protection (MF = 3.78/2.90 = 1.30; Fig. 4a). However after day 10 the baldness scores and time to full regrowth of both groups were nearly identical throughout the experimental period i.e., MF $\leq$ 1.

800 R: Since the baldness scores of WR-1065 treated animals receiving 800 R were maximal at 4.0 and were similar to those of saline controls, we used the "extrapolated" peak values to determine MFs. However there still were no differences between control or treated groups when extrapolated peaks (MF = 7/6.7 = 1.04), day of extrapolated peak (MF = 6.2/6.2 = 1) or day of full regrowth (MF = 27/26 = 1.04) were compared (Fig. 4b).

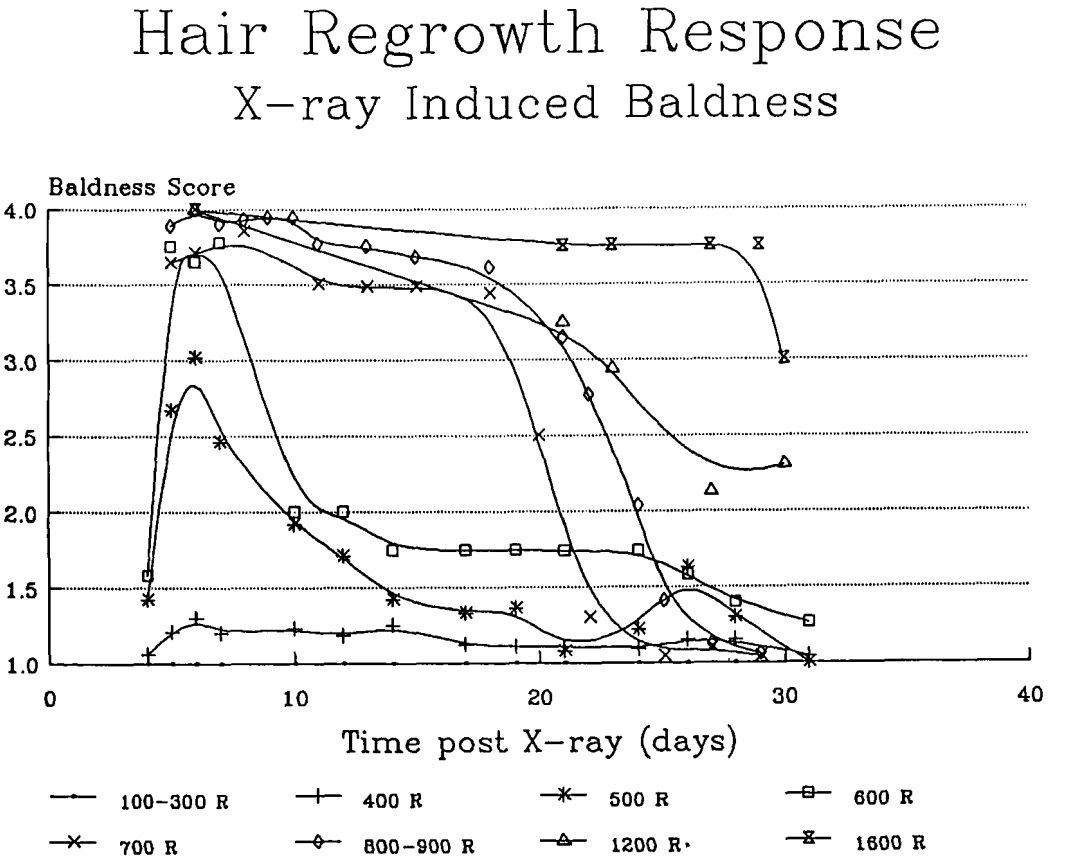

FIGURE 1

The mean baldness score of saline-treated control mice exposed to 100-1600 R X-rays at day 0 and evaluated for the next 30 days. Values for 0% regrowth (total baldness) = 4.0; 25% regrowth = 3; 50% regrowth = 2; 100% (full hair regrowth) = 1.0. Each data point represents the mean value from 4-11 animals. The SEMs ranged from 0-0.57 in all groups of animals analyzed and have been omitted for clarity in Figs 1-2.

WR-1065/DMF (Fig. 3; Table I)

600 R: Though the observed baldness peak score was less than controls (MF = 3.78/2.70 = 1.40; Fig. 4a) and represented a slight improvement (8%) when the MF was compared to WR-1065/saline, it was not statistically significant. The time to the occurrence of the peak was delayed (MF = day 10/day 7 = 1.43, a 100% increase compared to WR-1065/saline). The protected

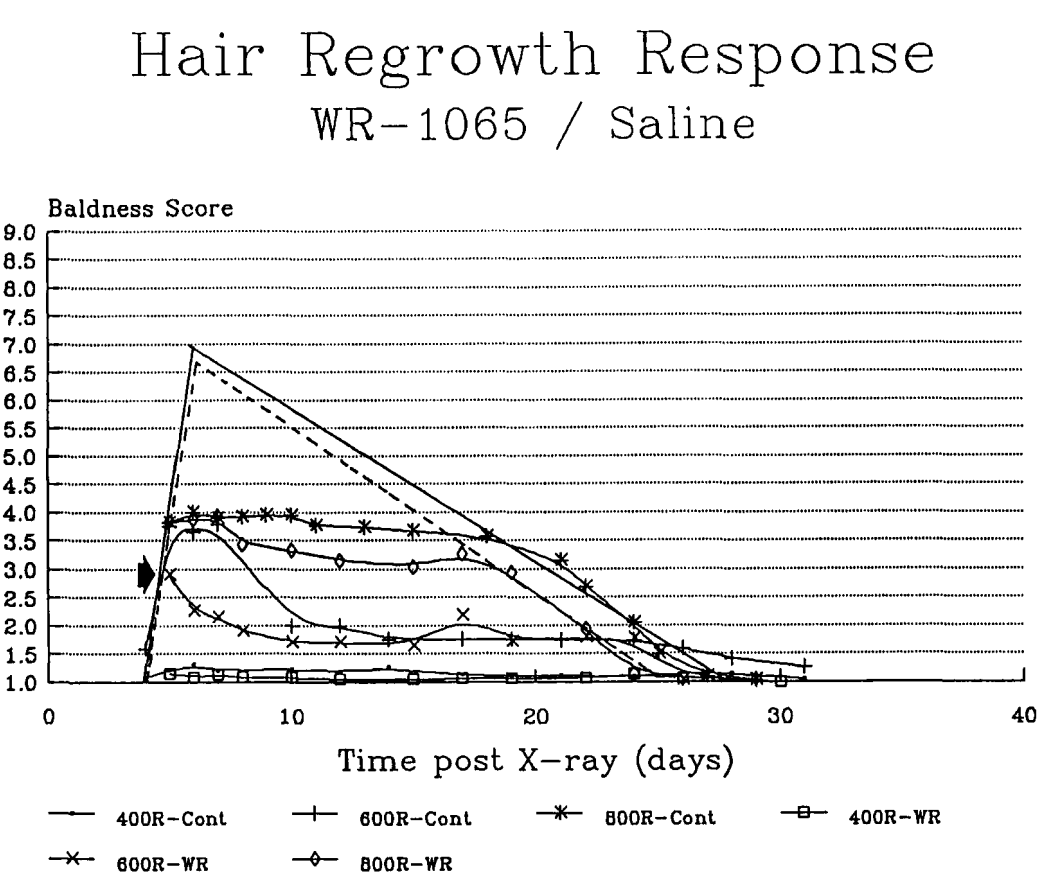

FIGURE 2

The hair regrowth response of mice treated topically with saline or WR-1065 in saline for 1 hr prior to X-irradiation with 400, 600 and 800 R. The mean baldness scores of the control animals were obtained from Fig. 1. Each point represents the mean from 5-6 animals. Extrapolated peaks of both groups exposed to 800 R were derived by fitting lines that best corresponded to the ascending and descending slopes of the response curves from control (solid line) and WR-treated (dotted lines). The arrow indicates a significant decrease in the peak baldness score ($P<0.005$) of protected animals compared with controls given 600 R.

animals also achieved an earlier full recovery (MF = 30/23 = 1.30, a 30% increase over WR-1065/saline).

800 R: The extrapolated peak baldness score in animals given WR-1065/DMF was lower than controls (MF = 7/5 = 1.40, a 35% improvement over WR-1065/saline) although this peak was not delayed (MF =

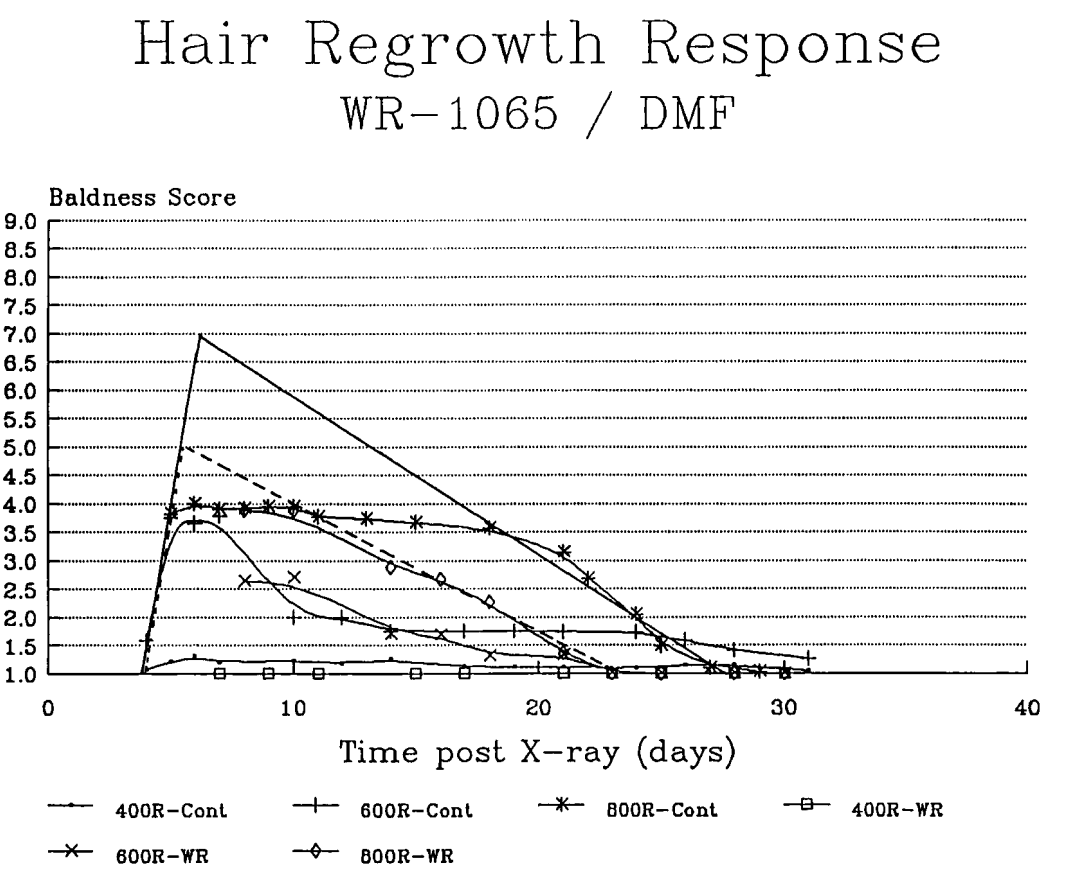

FIGURE 3

The hair regrowth response of mice treated with saline or WR-1065 in dimethyl formamide (DMF) for 1 hr prior to irradiation. Each point represents the mean values from 4-5 animals. Extrapolated peaks were obtained as described above.

5/6.2 = 0.81). The treated animals had full hair re-growth earlier than controls (MF = 27/23 = 1.17; a 13% improvement).

WR-1065/Propylene Glycol (Fig. 4; Table I)

600 R: The observed peak baldness score of treated mice was significantly lower ($P < 0.02$) than controls and the MF (3.78/2.63 = 1.44) was 10% better than WR-1065/saline. The day of the observed peak was delayed until day 17 (MF = 17/7 = 2.63; a 242%

TABLE 1

Percent Improvement of Modification Factors Using Permeation Enhancing Vehicles vs Saline

600 R	OBSERVED PEAK	TIME TO OBSERVED PEAK	TIME TO FULL RECOVERY
WR/DMF	8%	100%	30%
WR/PG	10%	242%	7%
CYST/PG	4%	N.I.[a]	N.I.
CYST/DMSO	12%	N.I.	N.I.
TEMPOL[b]	N.I.	N.I.	N.I.
800R	**EXTRAPOLATED PEAK**	**TIME TO EXTRAPOLATED PEAK**	**TIME TO FULL RECOVERY**
WR/DMF	35%	N.I.	13%
WR/PG	N.I.	N.I.	N.I.
CYST/PG	N.I.	N.I.	N.I.
CYST/DMSO	N.I.	N.I.	N.I
TEMPOL	N.I.	N.I.	N.I.

[a]No Improvement

[b]These animals had the fastest time from peak baldness to full hair regrowth (5 days) of any group given 600 R.

improvement over WR-1065/saline). However full hair regrowth in WR-treated mice was not achieved until day 28 (MF = 30/28 = 1.07; a 7% improvement).

800 R: Both WR-1065 treated and control groups had nearly identical baldness scores (3.9 - 4.0), extrapolated peaks (6.7 -7.0), and recovery times (Day 27). Therefore the MFs were trivial.

In summary, mice exposed to 600 R and treated by topical application of WR-1065 in any vehicle generally displayed protection as measured by diminished observed peak values and MFs greater than 1. The addition of WR-1065 to a permeation enhancing vehicle

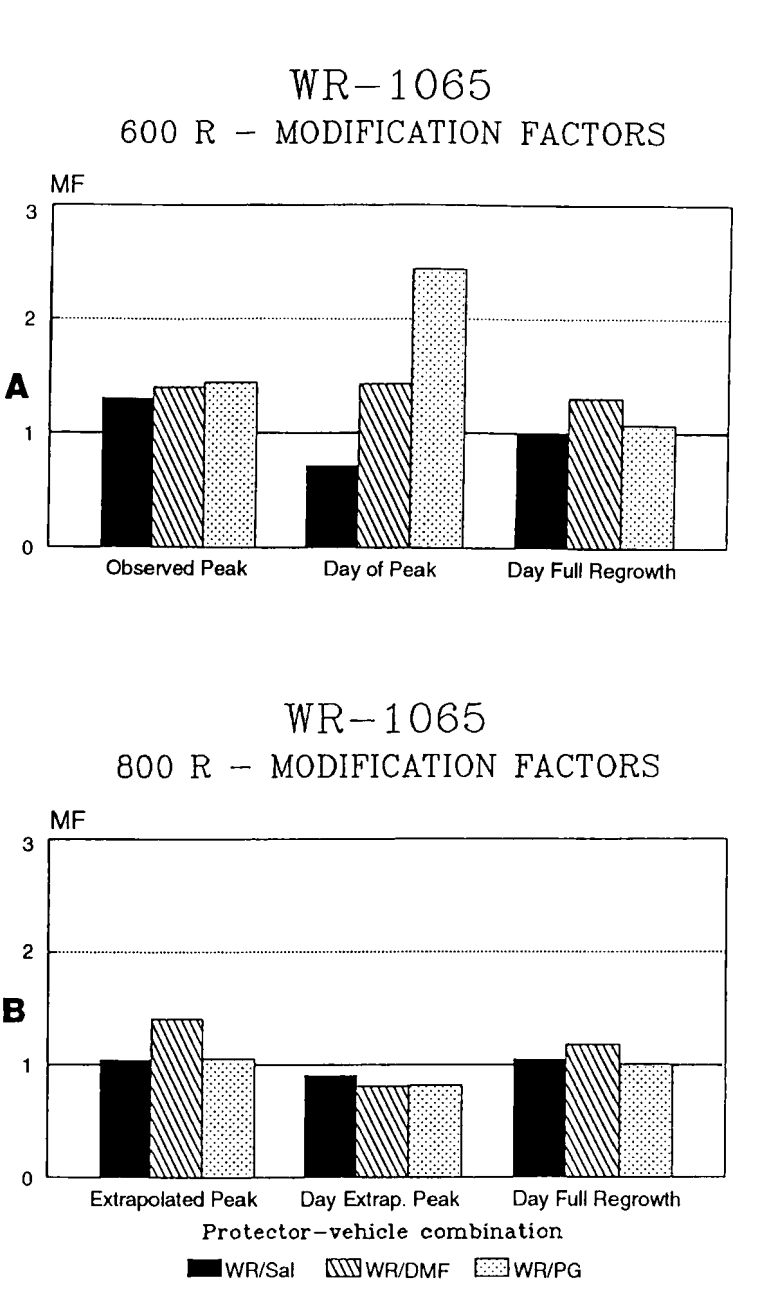

FIGURE 4

A) Modification factors of the observed peak, the day of the observed peak and day of full regrowth from mice treated with WR-1065 in saline, DMF or PG and irradiated with 600 R. **B)** Modification factors of the extrapolated peak, day of extrapolated peak and the day of full hair regrowth from mice treated with WR-1065 and the permeation enhancers described in Fig 4A and irradiated with 800 R.

resulted in MFs greater than those found in animals treated with WR-1065/saline in all parameters measured. The additional protective effects afforded by the use of enhancing vehicles ranged from 8-242%. When mice were exposed to 800 R, only WR-1065/DMF produced protection as seen by the reduction of the extrapolated peak.

Cysteine/Saline (Fig. 5)

600 R: In cysteine-treated animals the observed baldness score was significantly less than controls ($P < 0.001$; MF = 3.78/2.75 = 1.37;) but the day of the observed peak baldness score was identical (MF = 7/7 = 1).

800 R: Animals receiving cysteine/saline had identical baldness scores as control animals (3.7 - 4.0) for the first 12 days of the experimental period as well as similar extrapolated peaks (6.7 - 7.0) and days of the extrapolated peak (5.2 - 6.2). However the protected group had full hair regrowth by day 21 versus day 27 for controls (MF = 27/21 = 1.29).

Cysteine/Propylene Glycol (Fig. 5; Table I)

600 R: Though the day of the observed peak was nearly identical, the cysteine treated group showed a significant reduction in the observed peak baldness score ($P < 0.001$; MF = 3.78/2.66 = 1.42, only a 4% increase compared to mice treated with cysteine/saline) and achieved full hair recovery by day 28 (MF = 30/28 = 1.07).

800 R: The extrapolated peak (MF = 7/7.9 = 0.89) and the day of the peak (MF = 6.1/6.2 = 0.98) were not different in the control or cysteine-treated groups. The baldness scores in the treated group paralleled those of controls yet they did not achieve full recovery of hair regrowth by day 30 as did controls (MF <1).

Cysteine/DMSO (Fig. 5; Table I)

600 R: With this protector-vehicle combination improved protection was afforded at this dose. The observed peak baldness score for cysteine treatment was significantly less than controls ($P < 0.001$; MF = 3.78/2.46 = 1.54, 12% increase over the MF in the cysteine/saline group). Since baldness scores were essentially an extended flat peak throughout most of

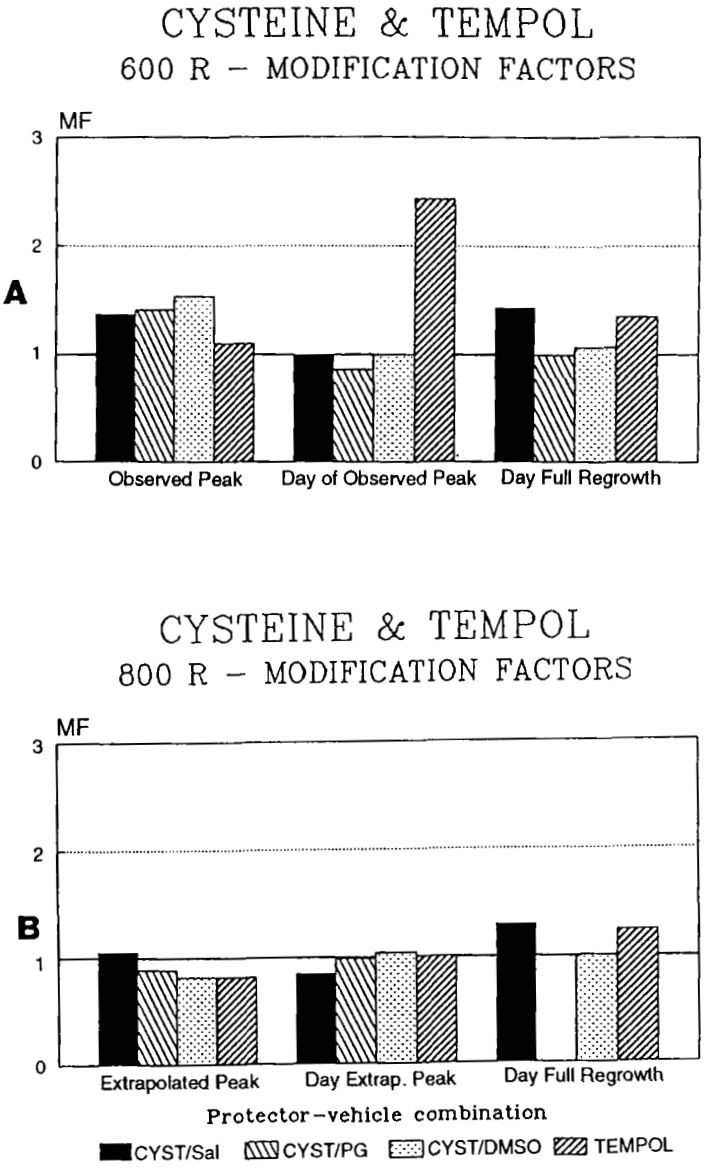

FIGURE 5

A) Modification factors of the observed peak, day of the observed peak and day of full regrowth from mice treated with cysteine in saline, PG or DMSO or with TEMPOL and irradiated with 600 R. **B)** Modification factors of the extrapolated peak, day of the extrapolated peak and the day of full hair regrowth from mice treated with the protector-vehicle combinations described above and irradiated with 800 R. Animals treated with cysteine in PG did not have full hair regrowth by the end of the observational period; hence a DMF of the day of full regrowth could not be calculated.

the observational period, we used day 7 as the day of the peak in calculating the MF (7/7 = 1). Full hair recovery occurred by day 28 (MF = 30/28 = 1.07).

800 R: The animals given cysteine/DMSO had nearly identical baldness scores as controls throughout the entire 30 day period. MFs based on extrapolated peaks and days of extrapolated peaks were minimal.

TEMPOL (Fig. 5; Table I)

600 R: In the TEMPOL-treated animals, the baldness scores rose slowly to a peak that was neither statistically significant from controls (MF = 3.78/3.43 = 1.1) nor better than cysteine/saline. After finally peaking at 17 days, full hair recovery then occurred rapidly, within 5 days, by day 22 (MF = 30/22 = 1.36). This was the fastest recovery time of any group.

800 R: Animals receiving a topical application of TEMPOL suffered an early (by day 5) and complete hair loss which persisted until day 18. However, hair regrowth was rapid and complete by day 22 whereas controls had full regrowth by day 27 (MF = 27/22 = 1.23). The extrapolated peak was higher in the TEMPOL-treated animals (MF = 7/8.5 = 0.82), but occurred at the same time as controls (MF = 6.2/6.2 = 1.0).

DISCUSSION

Hair loss is a significant physical and emotional side effect after therapeutic doses of ionizing radiation and/or chemotherapy. Since systemic administration of radioprotectors such as WR-2721 may have toxic effects, and possible tumor protective effects (6), that may preclude their clinical application (20), use of protectors in non-systemic strategies, eg. topically to the scalp, might capitalize on their protective effects while minimizing their negative attributes. However, the hydrophobic nature of the stratum corneum is thought to prevent significant passage of hydrophilic radioprotectors to the dividing cells in the underlying epidermis, dermis and hair follicles.

Skin has two layers: (a) a supporting dermis of vascularized connective tissue and (b) an overlying, stratified, avascular, cellular epidermis. The dermis supports hair follicles and sebaceous and sweat glands, which pass through pores in the epidermis to reach the surface. For hydrophilic drug penetration, the critical barrier in this multilaminated membrane

is the outer stratum corneum (horny layer), permitting only small quantities of topically applied substances to reach the dividing epidermal cells or to the radiosensitive targets in the underlying dermis (21). As a result of the barrier the greater part of applied drug remains on the surface and in the horny layer (22).

Transport of drugs through the skin may be 1) transepidermal (across the stratum corneum, inter- or intracellularly) or 2) via ducts of sweat glands or hair follicles (22). For a long time it was agreed that polar molecules pass the horny layer through the intracellular hydrated keratin of the outer dead cells; non-polar molecules were thought to traverse the intercellular lipids (22), the hydrophobic region of the lipid chains providing the non-polar route (24). Factors influencing the relative importance of various routes include physiochemical properties of the drug (25), time allowed for permeation, density of follicles and glands, thickness and hydration of the stratum corneum, and the modifying effects of the transporting vehicle on the skin (23).

Enhancers may pass into the skin decreasing its resistance to drug passage e.g., DMSO (26). A selection of vehicles, including glycols, sulfoxides, amides, amines and pyrrolidones have been evaluated in conjunction with drugs of varying polarities and partition coefficients for their abilities to enhance drug transport 27). The most crucial transport events occur in the lipid bilayer of the intercellular spaces of the stratum corneum but because of the structural complexity of skin drugs may use several pathways and in a dynamic fashion (27). The physical and chemical properties of the vehicle may alter the barrier and so determine the extent of drug entry into the skin. Ideal vehicles should adhere tightly to the skin surface and drugs should be largely or totally dissolved in it. Since drug solubility varies with each vehicle, a vehicle tailored for each drug is needed for optimal penetration. Additionally, penetration kinetics will depend on the state of hydration of the horny layer, the chemical nature of the drug and its manner of application (22), i.e., dose, vehicle, application time, anatomic site etc (28). These complex issues have never been adequately addressed with reference to the topical utilization of radioprotectors.

We earlier demonstrated that radiolabelled radioprotectors, when dissolved in suitable permeation enhancing vehicles, do indeed cross the stratum

corneum of the skin barrier into the underlying epidermal tissues and even access the vasculature of the dermis (8,9), better than when dissolved in water. We then needed to demonstrate if radioprotection occurs when these compounds are delivered topically. For the present study we chose the hair regrowth assay to measure protection since the "plucking" technique is known to stimulate the synchronous replication of cells in all of the affected hair follicles (12,13). These dividing cells would then be susceptible to X-ray damage, the degree of which could be subjectively measured (14).

Our first experiment demonstrated diminished hair regrowth or baldness occurring in a dose-dependent fashion, beginning at 400 R. In animals given more than 800 R, total baldness occurred and complete recovery of hair growth was not seen within the 30 day experimental period. On the basis of these results we chose 400, 600 and 800 R as suitable doses to be evaluated for protection.

Our earlier studies suggested that DMF and PG would be better vehicles than saline at promoting topical protector absorption (8). Irradiation with 400 R produced little effect in controls and this was not affected by application of any protectors in their vehicles, i.e. there was no radioprotection. At 600 R protection against "peak baldness" was obtained with WR-1065 in saline though slight improvement was achieved with the other vehicles (DMF and PG; 8-10%). When we evaluated by the "day of occurrence" of the peak baldness then DMF and PG were increasingly protective (100-242%); full regrowth was improved by DMF only. At 800 R WR-1065 in DMF appeared to be more effective than when in saline or PG when "extrapolated peaks" (35%) or day of complete hair regrowth (13%) were analyzed. Thus, judged by certain parameters of protection, WR-1065, the dephosphorylated form of WR-2721, did enhance protection when topically applied with saline and with other permeation enhancing vehicles. In general WR-1065 in saline was slightly protective, consistent with a recent study by Geng et al (29) that showed protection of hair growth with topical application of WR-1065 in saline after fractionated doses of X-rays. There was a variable increase in protection when DMF or PG replaced saline.

Since there was protection when saline was the vehicle and only a slight improvement when the proven skin permeation-enhancing vehicles DMF and PG (8) were used, it seems that enhanced passage across the layers

of epidermal cells was not attained. Rather, all of our protector-vehicle combinations may have additionally taken the more direct transfollicular route to reach the dividing cells in the hair follicle. As Williams and Barry (3) have suggested, if these penetrants took the transepidermal route, the concentration would be near zero in the dermis anyway due to the efficient sink conditions provided by the underlying vasculature. Using this rationale, our protector-vehicle combinations would be taken up by the dermal capillaries and greatly diluted in the general body vasculature before ever reaching the hair papillae. Thus we believe that the transfollicular route may be the most likely and pertinent path utilized in these experiments.

Regarding cysteine and TEMPOL, at 400 R the effect was again so slight that no appreciable differences could be measured between control and treated animals. At 600 R all cysteine-vehicle combinations produced protection i.e., reduced the observed peak baldness score (MFs >1) while TEMPOL did not. Relative to the delay in the time of occurrence of observed peak baldness scores only TEMPOL and cysteine in DMSO were able to produce substantial MFs (> 2.0). Interestingly, TEMPOL produced the fastest recovery time between peak baldness score and full hair regrowth (5 days; Fig 8). These data corroborate those of Goffman et al (11) who demonstrated the radioprotective effects of TEMPOL in enhancing hair regrowth in the guinea pig. Little or no protection was seen by any of our parameters at the 800 R dose when cysteine or TEMPOL was used as the protecting compound.

Our earlier reports in the rat (9) and mouse (8) suggested that better and differential penetration of radioprotectors could be obtained when combined with permeation enhancing compounds other than saline. This led to the hypothesis that protection might occur better after topical application with vehicles other than saline. The results of the current study indicate that as a vehicle for WR-1065, saline itself allows some protection and that utilizing vehicles which actually enhance protector penetration better than saline did, though not markedly, improve the radiation induced baldness outcome.

While vehicles alone were not tested for protective value, our data suggest that in most cases they did not play a role in protecting the dividing cells in the hair follicle. For example, animals

treated with cysteine in any vehicle other than saline and exposed to 600 R and 800 R had longer recovery times to full hair regrowth, i.e., were more damaged. If DMSO, said to be a radioprotector (15), DMF or PG were protective themselves, then we would have observed earlier recovery times with these protector-vehicle combinations than with saline.

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