Pergamon

Int. J. Radiation Oncology Biol. Phys., Vol. 32, No. 4, pp. 1047–1052, 1995

0360-3016/95 $9.50 + .00

0360-3016(94)00467-6

● *Biology Original Contribution*

ACEMANNAN-CONTAINING WOUND DRESSING GEL REDUCES RADIATION-INDUCED SKIN REACTIONS IN C3H MICE

DIANNA B. ROBERTS, PH.D. AND ELIZABETH L. TRAVIS, PH.D.

Department of Experimental Radiotherapy, The University of Texas M.D. Anderson Cancer Center, Houston, TX 77030

Purpose: To determine (a) whether a wound dressing gel that contains acemannan extracted from *aloe* leaves affects the severity of radiation-induced acute skin reactions in C3H mice; (b) if so, whether other commercially available gels such as a personal lubricating jelly and a healing ointment have similar effects; and (c) when the wound dressing gel should be applied for maximum effect.

Methods and Materials: Male C3H mice received graded single doses of gamma radiation ranging from 30 to 47.5 Gy to the right leg. In most experiments, the gel was applied daily beginning immediately after irradiation. To determine timing of application for best effect, gel was applied beginning on day −7, 0, or +7 relative to the day of irradiation (day 0) and continuing for 1, 2, 3, 4, or 5 weeks. The right inner thigh of each mouse was scored on a scale of 0 to 3.5 for severity of radiation reaction from the seventh to the 35th day after irradiation. Dose–response curves were obtained by plotting the percentage of mice that reached or exceeded a given peak skin reaction as a function of dose. Curves were fitted by logit analysis and ED_{50} values, and 95% confidence limits were obtained.

Results: The average peak skin reactions of the wound dressing gel-treated mice were lower than those of the untreated mice at all radiation doses tested. The ED_{50} values for skin reactions of 2.0–2.75 were approximately 7 Gy higher in the wound dressing gel-treated mice. The average peak skin reactions and the ED_{50} values for mice treated with personal lubricating jelly or healing ointment were similar to irradiated control values. Reduction in the percentage of mice with skin reactions of 2.5 or more was greatest in the groups that received wound dressing gel for at least 2 weeks beginning immediately after irradiation. There was no effect if gel was applied only before irradiation or beginning 1 week after irradiation.

Conclusion: Wound dressing gel, but not personal lubricating jelly or healing ointment, reduces acute radiation-induced skin reactions in C3H mice if applied daily for at least 2 weeks beginning immediately after irradiation.

Acemannan, Acute skin reaction, Radioprotectors.

INTRODUCTION

The limiting factor in radiotherapy of tumors is damage to surrounding normal tissue. Because the skin is included in the target radiation field in external-beam treatment protocols for breast cancer, an agent that could reduce the severity of radiation-induced skin reactions would be a useful adjunct to such treatment. Collins and Collins (5) described the use of *aloe vera* gel for treatment of radiodermatitis in 1935. Since then a number of investigators have attempted to treat various types of radiation-induced skin reactions with either fresh gel or various ointment preparations of *aloe* (1, 14, 15, 28). Although the results of these studies were mixed, it seems that the fresh gel, but not the ointment preparations, reduced the healing time of the lesions slightly.

For many years, pulp from the *aloe vera* plant has been reported to be useful in the healing of sunburns, scalds, and minor cuts and abrasions. More recently, published experiments have documented its beneficial effects in these same types of dermal injuries (1, 7, 8, 10, 11, 20). In many of the earlier studies that described accelerated healing with application of *aloe vera*, the freshly har-

Presented at the Sixth International Symposium on Wound Healing and Wound Management, sponsored by the International Burn Foundation, San Francisco, CA, 9–10 October, 1993.

Reprint requests to: Elizabeth L. Travis, Ph.D., The University of Texas M. D. Anderson Cancer Center, 1515 Holcombe Blvd., Box 66, Houston, TX 77030-4095.

Acknowledgements— We thank Lane Watkins and his staff for the supply and care of the animals used in this study; Virginia Watts and Meredith Worthen, King Foundation Scholars, for their assistance in some of these experiments; and Barbara Bickerstaff for preparation of the manuscript. Wound dressing gel was generously supplied by Dr. Kenneth M. Yates of Carrington Laboratories, Inc. This work was supported in part by grants from Carrington Laboratories, Inc. and CA06294 from the National Cancer Institute, Department of Health and Human Services.

Accepted for pbulication 19 August 1994.

vested pulp from the plant was used. In some later studies, preserved extracts from the pulp were used and found variably effective (1, 6-8, 11, 13, 26). The practical advantages of being able to use a relatively stable commercially available preparation rather than relying on freshly harvested material of unknown potency seem obvious. More recently, extracts of *aloe barbadensis* produced by a commercial manufacturer[1] have been tested and found to accelerate healing of surgical wounds and burns, as well as demonstrate antitumor effects (12, 18, 19, 22, 23).

In light of the published reports on the efficacy of wound dressing gel[2] in reducing healing time of surgical wounds and thermal burns, we thought it worthwhile to examine whether this preparation has an effect on acute radiation-induced skin reactions. In particular, we sought to determine (a) whether application of this gel would alter the severity or duration of these skin reactions in mice; (b) whether other gels not containing components from *aloe* pulp might have similar effects; and (c) what was the optimum timing of gel application for best effect.

MATERIALS AND METHODS

Mice

Male C3Hf/Kam mice, 11–13 weeks of age at the time of irradiation, were used in these experiments. The mice were bred and kept in a barrier-maintained, specific pathogen-free, defined flora facility approved by the American Association for Accreditation of Laboratory Animal Care and in accordance with current regulations and standards of the U.S. Department of Agriculture and Department of Health and Human Services, National Institutes of Health. Mice were housed in groups of five in sterilized polycarbonate cages with filter bonnets. They were fed autoclaved feed and sterile acidified water ad lib. The photoperiod was regulated to a 12 h light/12 h dark cycle.

Radiation

To assess the effects of the various gels on radiation-induced skin reactions, unanesthetized mice were restrained in special jigs with only the right hind thighs of the mice exposed to the beam. The mouse legs were irradiated by a dual-source ^{137}Cs gamma-ray source at a dose rate of 7.07 Gy/min. The mice were kept in perforated chambers through which 4–6 l pm 100% O_2 flowed for 15 min before and during irradiation.

Gels

Wound dressing gel[3] was generously supplied in tubes by a commercial manufacturer. Constituents listed on the label were: purified water, povidone, panthenol, Carbomer 940, triethanolamine, allantoin, glutamic acid, sodium chloride, methylparaben, imidazolidinyl urea, sodium benzoate, potassium sorbate, acemannan hydrogel, citric acid, and sodium metabisulfite. Personal lubricating jelly[4] was compared with wound dressing gel with respect to its effects on radiation-induced skin reactions because it is a water-soluble hydrogel, similar in solubility and consistency to wound dressing gel. According to its label, personal lubricating jelly contained: chlorhexidine gluconate, glucono delta lactone, glycerin, hydroxyethyl cellulose, methylparaben, purified water, and sodium hydroxide. Healing ointment[5] was compared with wound dressing gel because it was currently being distributed to patients undergoing radiotherapy at The University of Texas M. D. Anderson Cancer Center. Its label listed petrolatum, mineral oil, mineral wax, wool wax alcohol, pantothenol, glycerin, and bisabolol (chamomile essence) as ingredients. No gel similar in constitution to wound dressing gel but without acemannan or allantoin was available for comparison with wound dressing gel.

The gels were liberally applied to the irradiated mice legs with a gloved index finger. Care was taken not to touch the irradiated leg with the finger but only with gel. Mice in gel treatment groups were kept on plastic platforms above the bedding so that bedding particles did not adhere to the treated legs.

Assay of Skin Damage

The ventral side of the right thigh of each irradiated mouse was scored daily from the seventh to the 35th day after irradiation on a scale of 0 (no visible reaction) to 3.5 (breakdown of the skin over the entire radiation field with severe exudation) according to the system described by Masuda *et al.* (16, 17). Intermediate scores were assigned as appropriate. Scoring was done before gel application each day.

Experimental Design

In experiments designed to determine whether wound dressing gel affected either the severity or the duration of radiation-induced skin reactions, groups of 20–40 C3H mice received graded single doses of gamma rays ranging from 30 to 47.5 Gy to the right thigh. Beginning immediately after irradiation and continuing daily for the entire observation period, wound dressing gel was applied to half of the mice in each dose group. The other mice were left untreated. Skin reactions were scored as described above.

Dose–response curves were obtained by plotting the percentage of mice that reached or exceeded a given peak skin reaction (e.g., 2.5) as a function of dose. These curves

[1] Carrington Industries, Irving, TX.
[2] Carrington Gel Wound Dressing™.
[3] Carrington Gel Wound Dressing™, Carrington Laboratories, Inc., Irving, TX.
[4] K-Y® Brand Jelly, Johnson and Johnson Consumer Products, Inc., Skillman, NJ.
[5] Aquaphor® Healing Ointment, Beirsdorf, Inc., Norwalk, CT.

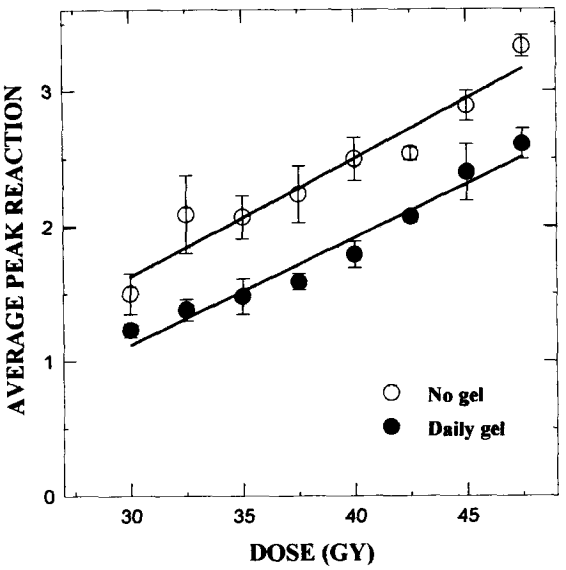

Fig. 1. Average peak skin reactions on inner thighs of mice as a function of single doses of x-rays either alone (○) or with daily application of wound dressing gel for 35 days beginning immediately after irradiation (●). Each point represents the mean of peak scores from 20–50 mice in two to five separate experiments. Error bars are standard errors of the means. Regression lines were fitted by least squares analysis.

were fitted by logit analysis, and ED_{50} values and 95% confidence limits were obtained. Data from two to five experiments were analyzed separately. No differences between the experiments were found and the data from all experiments were combined in determining average peak reactions and percent responder mice as functions of dose.

To compare the effects of personal lubricating jelly and healing ointment with those of wound dressing gel in modifying the severity and/or duration of radiation-induced skin reactions, groups of 15 C3H mice received single doses of 35, 40, or 45 Gy to the right thigh followed immediately by treatment with wound dressing gel, personal lubricating jelly, healing ointment, or nothing. Gel applications continued daily throughout the observation period. Skin reactions were scored as described above.

To determine the timing of wound dressing gel application for optimal effects on radiation-induced skin reactions, groups of 10–20 mice received their first application of gel at day −7, day 0, or day +7 relative to the day of irradiation (day 0; 45 Gy). Gel application was repeated daily for 1, 2, 3, 4, or 5 weeks, depending on the experimental group. Skin reactions were scored as described above.

RESULTS

The peak skin reactions of individual mice in each dose group after single radiation doses alone or followed by daily application of wound dressing gel were averaged and plotted as a function of dose (Fig. 1). At all radiation doses tested, the gel-treated mice exhibited lower average peak reactions than did the mice that received no gel. Also, because the maximum reactions were lower for the gel-treated mice and the reactions of both gel-treated and irradiated-only mice began to subside at about the same time, the reactions of the gel-treated mice reached the "healed" score of 1.0, at which the skin is once more intact, more quickly than did those of the mice that were irradiated only.

Figure 2 shows dose–response curves for the percentage of gel-treated mice or mice that were only irradiated that equaled or exceeded a reaction level of 2.5 (breakdown of about 50% of the skin in the radiation field). There is a clear displacement of the dose–response curve for skin reaction in gel-treated mice to the right of those only irradiated, giving ED_{50} values of 45.44 Gy (95% confidence limits = 44.1 Gy, 47.5 Gy) and 35.8 Gy (95% confidence limits = 37.2 Gy and 39.7 Gy), respectively. Table 1 lists the ED_{50} values and 95% confidence limits for other levels of peak skin reaction, ranging from 2.0 (breakdown of 10–20% of the skin in the radiation field) to 2.75 (breakdown of 67–75% of the skin in the field) for gel-treated and irradiated-only mice. At these thresholds, which encompass moderate to moderately severe skin reaction levels, the ratios of the ED_{50} values for the radiation-only mice vs. the radiation plus gel-treated mice were about 1.2.

Figure 3 shows average peak reactions as a function of dose for groups of mice treated daily with wound dressing gel, personal lubricating jelly, healing ointment, or nothing after selected single doses of radiation. The mice treated with wound dressing gel exhibited lower peak

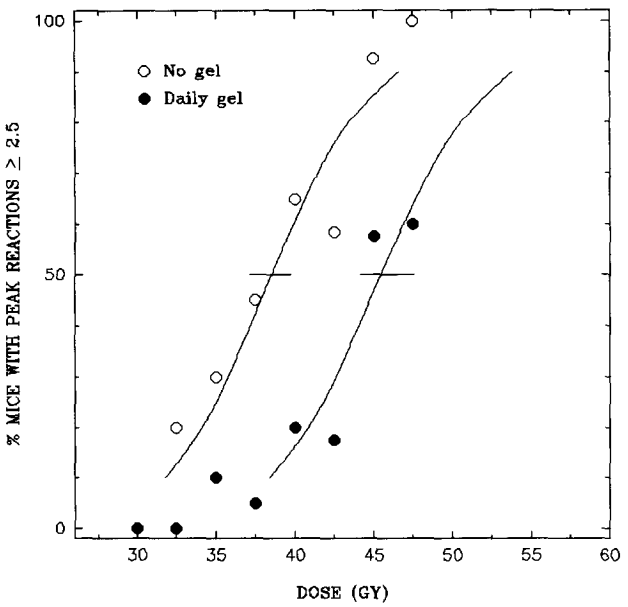

Fig. 2. Data from Fig. 1 expressed as the percentage of mice that exhibited peak skin reactions of 2.5 or higher after single doses of x-rays alone (○) or with daily application of gel for 35 days beginning immediately after irradiation (●). Lines were fitted by logit analysis of the data with 95% confidence limits shown at the ED_{50} (values given in Table 1).

Table 1. ED_{50}'s for radiation-induced skin reactions in C3H mice with and without daily application of wound dressing gel

Threshold reaction level	No gel ED_{50} (Gy) (95% c.l.)	Daily gel ED_{50} (Gy) (95% c.l.)
2.0	31.90 (30.4–33.0)	38.46 (37.1–39.7)
2.25	36.21 (35.0–37.3)	43.54 (42.2–44.9)
2.5	38.50 (37.2–39.7)	45.44 (44.1–47.5)
2.75	40.91 (39.7–42.1)	48.06 (46.1–52.7)

reactions at all three radiation doses tested than did the mice in any other treatment group.

To determine the optimum time of administration of wound dressing gel, eight groups of mice were treated with a single dose of 45 Gy (day 0) and gel was applied for various times before or after irradiation. The percentage of mice in each treatment group that reached or exceeded peak skin reaction levels of 2.25, 2.5, and 3.0 were determined. As can be seen from the percentage of mice that reached 2.5 or higher (Fig. 4), the largest reduction in peak skin responses occurred when the gel was applied daily for at least 2 weeks beginning immediately after irradiation. If application of the gel was delayed until 1 week after irradiation, which is well before the onset of a detectable skin reaction, there was no reduction in the severity of peak skin responses. Application of gel from 1 week before to the day of irradiation also was ineffective.

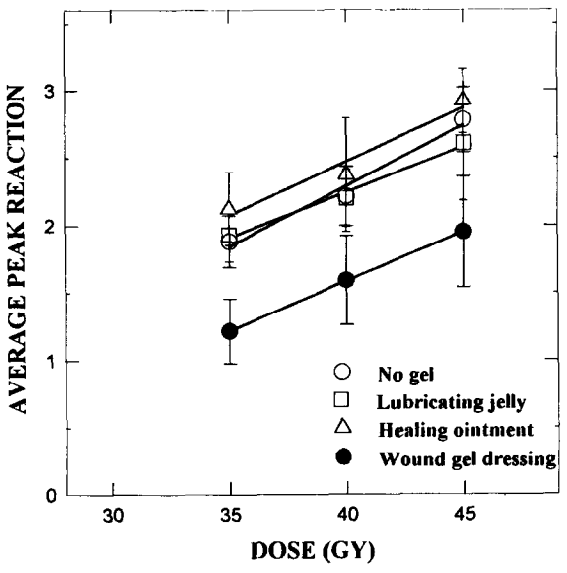

Fig. 3. Average peak skin reactions of mice as a function of single doses of x-rays either alone (○), or with daily application of personal lubricating jelly (□), healing ointment (△), or wound dressing gel (●) for 35 days beginning immediately after irradiation. Each point represents the mean of peak scores from groups of 15 mice. Error bars are standard deviations from the means. Regression lines were fitted by least squares analysis.

DISCUSSION

The results of these experiments indicate that application of wound dressing gel reduces the severity of acute radiation-induced skin reactions in C3H mice. Because the reactions are less severe in gel-treated mice, such mice also recover faster from this type of injury. Unlike previously studied *aloe*-based ointments, wound dressing gel maintains the healing properties associated with the fresh pulp of the plant but in a stable form with standardized potency. Two other moisturizing, non-*aloe*-based preparations, personal lubricating jelly and healing ointment, had no such effect when applied under similar conditions, even though the latter is used clinically. For best effect, wound dressing gel should be applied daily for 2 or 3 weeks beginning immediately after irradiation.

Although we only considered topical gels in our study, we did not ignore the potential uses of systemic radioprotective agents in reducing radiation-induced skin reactions. The well-known radioprotective agent WR 2721 (S-2-(3-aminopropyl-amino) ethyl phosphorothioate) has been shown by a number of investigators to reduce acute skin reactions in irradiated mice (9, 21, 25, 30, 31). Travis et al. (24) reported dose-modifying factors ranging from 1.1 to 2.1 depending on how long before irradiation the drug was injected, whether the animals were irradiated in O_2 or air, and whether the drug was injected intravenously or intraperitoneally. To be effective, this agent, thought to exert its radioprotective effect largely through free radical scavenging, must be injected before each fraction of the radiation treatment. However, WR 2721 has not been useful clinically because it induces hypertension (3, 29). Conversely, the wound dressing gel used in this study exerted a significant radioprotective effect when applied topically, not systemically, with no observable toxic effects. This would argue in favor of its being a useful clinical adjunct to radiotherapy, provided it has similar properties in humans.

The mechanism behind the meliorative effect of the wound dressing gel is probably not simply a mechanical one of maintaining moisture at the site of the lesion. The wound dressing gel-treated mouse legs appeared dry within an hour of treatment. The personal lubricating jelly is a watersoluble gel very similar in consistency to wound dressing gel, and thus would likely have similar moisturizing properties, but its application had no effect on the severity of the skin reactions. Healing ointment is a petroleum wax-based product that would be expected to retain moisture well and is described on its label as "clinically proven to reduce wound healing time," but it also did not affect the radiation-induced skin reactions. One of the constituents of wound dressing gel is acemannan hydrogel. Acemannan is a highly acetylated β1,4 polymer of mannose, which induces secretion of several cytokines,

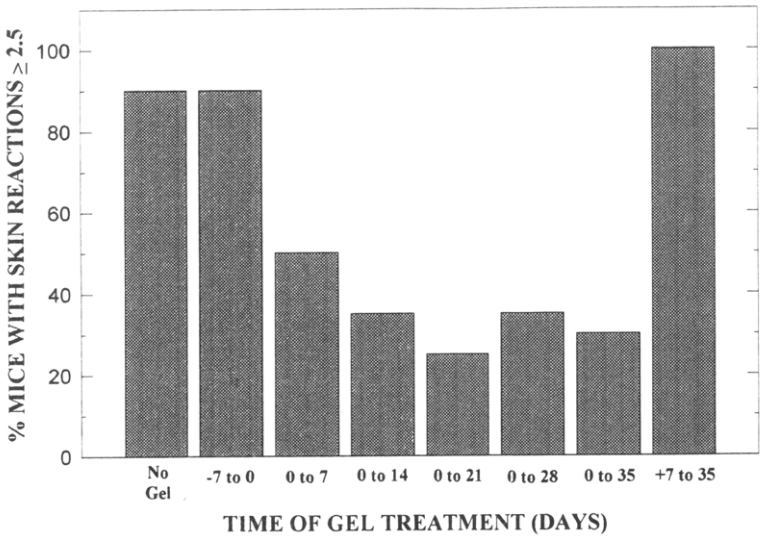

Fig. 4. Percentage of mice that exhibited peak skin reactions of 2.5 or higher as a function of timing of application of wound dressing gel. Each bar represents responses of 10–20 mice.

including tumor necrosis factor and interleukin-1 (18, 27). Some of these cytokines are thought to regulate wound healing (2, 4) and might also affect acute radiation injury. However wound dressing gel exerts its effect, it would seem to affect the mechanisms involved in the induction of the skin reaction rather than in the later, healing phase. This hypothesis is supported by our observations that (a) the gel must be applied immediately (or very soon) after irradiation to be effective; (b) that if its application is delayed by a week, even though the mildest manifestations of the reaction do not occur until 10–15 days after irradiation, there is no reduction in peak reaction levels; and (c) that the gel does not reduce healing time of reactions of a given severity once they have occurred. The effect we observed is solely that of reducing the severity of the reactions induced by radiation.

REFERENCES

1. Ashley, F. L.; O'Loughlin, B. J.; Peterson, R.; Fernandez, L.; Stein, H.; Schwartz, A. N. The use of *Aloe vera* in the treatment of thermal and irradiation burns in laboratory animals and humans. Plast. Reconst. Surg. 20:383–396; 1957.
2. Barbul, A. Immune aspects of wound repair. Clin. Plast. Surg. 17:433–442; 1990.
3. Caldwell, R. W.; Heiffer, M. H. Acute cardiovascular and autonomic effects of WR-2721: A radioprotective compound. Radiat. Res. 62:62–69; 1975.
4. Coleman, C. N. Beneficial liaisons: Radiobiology meets cellular and molecular biology. Radiother. Oncol. 28:1–15; 1993.
5. Collins, C. E.; Collins, C. Roentgen dermatitis treated with fresh whole leaf of *Aloe vera*. Am. J. Roentg. Rad. Ther. 33:396–397; 1935.
6. Davis, R. H.; Kabbani, J. M.; Maro, N. P. *Aloe vera* and wound healing. J. Am. Podiatr. Med. Assoc. 77:165–169; 1987.
7. Davis, R. H.; Leitner, M. G.; Russo, J. M.; Byrne, M. E. Wound healing: Oral and topical activity of *Aloe vera*. J. Am. Podiatr. Med. Assoc. 79:559–562; 1989.
8. Davis, R. H.; Rosenthal, K. Y.; Cesario, L. R.; Rouw, G. A. Processed *Aloe vera* administered topically inhibits inflammation. J. Am. Podiatr. Med. Assoc. 79:395–397; 1989.
9. Echols, F. S.; Yuhas, J. M. Chemoprotection against fractionated radiation exposures with WR-2721: Skin injury. Radiat. Res. 66:499–504; 1976.
10. El Zawahry, M.; Hegazy, M. R.; Helal, M. Use of *Aloe* in treating leg ulcers and dermatoses. Int. J. Dermatol. 12:68–73; 1973.
11. Fulton, J. E., Jr. The stimulation of postdermabrasion wound healing with stabilized *Aloe vera* gel-polyethylene oxide dressing. J. Dermatol. Surg. Oncol. 16:460–467; 1990.
12. Harris, C.; Pierce, K.; King, G.; Yates, K. M.; Hall, J.; Tizard, I. Efficacy of acemannan in treatment of canine and feline spontaneous neoplasms. Mol. Biother. 3:207–213; 1991.
13. Kaufman, T.; Kalderon, N.; Ullmann, Y.; Berger, J. *Aloe vera* gel hindered wound healing of experimental second-degree burns: A quantitative controlled study. J. Burn Care Rehab. 9:156–159; 1988.
14. Loveman, A. B. Leaf of *Aloe vera* in treatment of roentgen ray ulcers. Arch. Dermatol. Syph. 36:838–843; 1936.
15. Lushbaugh, C. C.; Hale, D. B. Experimental acute radiodermatitis following beta irradiation. Cancer 6:690–698; 1953.
16. Masuda, K. Effects of some physiological conditions on the radiosensitivity of mouse skin. Nippon Acta Radiol. 39:884–889; 1979.
17. Masuda, K.; Matsuura, K.; Withers, H. R.; Hunter, N. Response of previously irradiated mouse skin to a second course of irradiation: Early skin reaction and skin shrinkage. Int. J. Radiat. Oncol. Biol. Phys. 12:1645–1651; 1986.
18. Peng, S. Y.; Norman, J.; Curtin, G.; Corrier, D.; McDaniel, H. R.; Busbee, D. Decreased mortality of Normal Murine Sarcoma in mice treated with the immunomodulator, Acemannan™. Mol. Biother. 3:79–87; 1991.
19. Rodríguez-Bigas, M.; Cruz, N. I.; Suárez, A. Comparative

evaluation of *Aloe vera* in the management of burn wounds in guinea pigs. Plast. Reconst. Surg. 81:386–389; 1988.
20. Rowe, T. D.; Lovell, B. K.; Parks, L. M. Further observations on the use of *Aloe vera* leaf in the treatment of third degree X-ray reactions. J. Am. Pharmaceut. Assoc. 30:266–269; 1941.
21. Stewart, F. A.; Rojas, A. Radioprotection of mouse skin by WR-2721 in single and fractionated treatments. Br. J. Radiol. 55:42–47; 1982.
22. Thompson, J. E. Topical use of *Aloe vera* derived allantoin gel in otolaryngology. (Letter to the Editor). Ear Nose Throat J. 70:56; 1991.
23. Tizard, I. R.; Carpenter, R. H.; McAnalley, B. H.; Kemp, M. C. The biological activities of mannans and related complex carbohydrates. Mol. Biother. 1:290–296; 1989.
24. Travis, E. L.; De Luca, A. M.; Fowler, J. F.; Padikal, T. N. The time course of radioprotection by WR-2721 in mouse skin. Int. J. Radiat. Oncol. Biol. Phys. 9:843–850; 1982.
25. Utley, J. F.; Phillips, T. L.; Kane, L. J. Protection of normal tissues by WR2721 during fractionated irradiation. Int. J. Radiat. Oncol. Biol. Phys. 1:699–703; 1976.
26. Watcher, M. A.; Wheeland, R. G. The role of topical agents in the healing of full-thickness wounds. J. Dermatol. Surg. Oncol. 15:1188–1195; 1989.
27. Womble, D.; Helderman, J. H. Enhancement of allo-responsiveness of human lymphocytes by acemannan (Carrisyn™. Int. J. Immunopharmac. 10:967–974; 1988.
28. Wright, C. S. *Aloe vera* in the treatment of roentgen ulcers and telangiectasis. JAMA 106:1363–1364; 1936.
29. Yuhas, J. M.; Proctor, J. O.; Smith, L. H. Some pharmacological effects of WR-2721: Their role in toxicity and radioprotection. Radiat. Res. 54:222–233; 1973.
30. Yuhas, J. M.; Spellman, J. M.; Culo, F. The role of WR-2721 in radiotherapy and/or chemotherapy. Cancer Clin. Trials 3:211–216; 1980.
31. Yuhas, J. M.; Storer, J. B. Differential chemoprotection of normal and malignant tissues. J. Natl. Cancer Inst. 42:331–335; 1969.