Wound Healing

The Effects of Topical Antimicrobial Agents

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• The effect of four commonly used topical antimicrobial agents on the rate of reepithelialization of clean wounds was evaluated in white domestic pigs. Neosporin Ointment was found to significantly increase the rate of reepithelialization by 25%, while Furacin significantly retarded the healing rate by 24%. Pharmadine, a preparation containing povidone-iodine, did not affect the rate of healing. Both Silvadene and its vehicle significantly increased the rate of reepithelialization by 28% and 21%, respectively. The effects of these agents cannot be explained on the basis of their antimicrobial activity.

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Topical antimicrobial agents have been widely used by dermatologists, surgeons, and family practitioners to prevent bacterial contamination of wounds. There is a wealth of information concerning the antibacterial efficacy of topical agents.[1-4] However, there are few published data concerning the effects of these agents on the rates at which wounds heal.

Perhaps one of the reasons for the paucity of information on the subject has been the difficulty in determining when a wound is healed. Reepithelialization has been widely held as a suitable end point.[5-7] Evaluation of reepithelialization has been accomplished by various means. Gruber et al[8] chose the presence of a pink surface without a scab as an indication of complete epithelialization, while Winter measured epidermal regeneration by examining serial histologic sections.[5] A new method for assessing reepithelialization of a wound, developed by Eaglstein and Mertz,[9] provides an objective and rapid means for evaluating the rate of epidermal wound healing. With this method we have evaluated the effects of four commonly used topical antimicrobial agents on epidermal wound healing. These agents were Neosporin Ointment (Burroughs Wellcome, Research Triangle Park, NC), Pharmadine (Sherwood Pharmaceutical Co, Mahawah, NJ), Furacin (Norwich-Eaton Pharmaceuticals, Norwich, NY), and Silvadene cream (Marion Labs, Kansas City, Mo). The agents were applied separately to multiple wounds on white domestic pigs. When statistically significant differences were found between an active agent and the untreated control, the active agent was compared with its vehicle. White domestic pigs were used because of the similarity of their skin to human skin.[10,11]

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MATERIALS AND METHODS

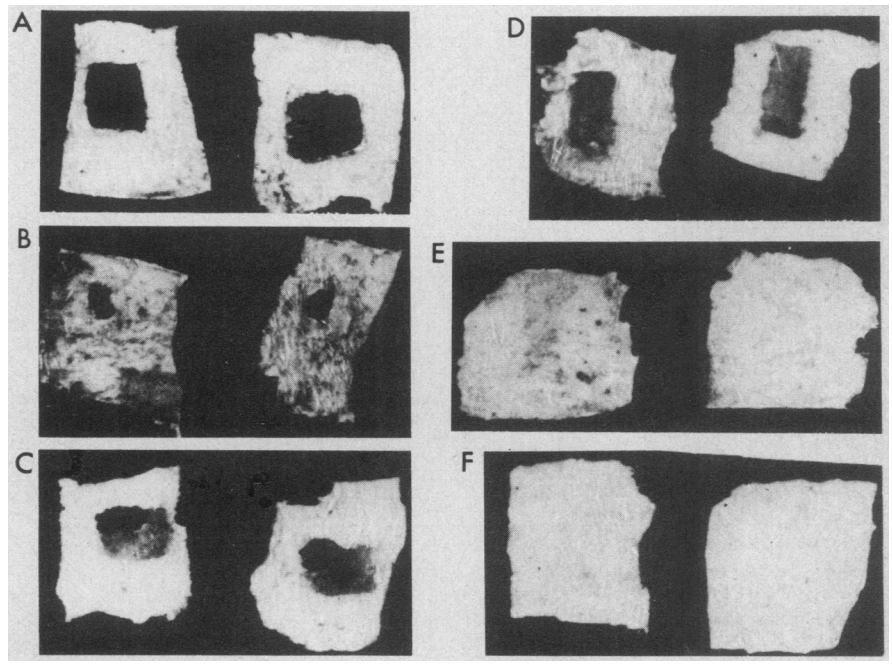
Young, white domestic pigs weighing 5.5 to 9.1 kg were shaved with standard animal clippers; the remaining hairs were shaved with barber's clippers. Each experimental animal was anesthetized with 15 mg/kg of pentobarbital sodium and approximately 150 rectangular wounds measuring 7 × 10 mm, 0.3 mm deep, were made over the vertebral and thoracic areas with a Castroviejo dermatome. The wounds were separated by approximately 15 mm. Seventeen experimental animals were used in this study, and they were housed individually in bins. Five animals received Neosporin Ointment and its vehicle and four animals were treated with each of the other agents tested and their vehicles.

Sampling and Direct Evaluation

On days 2 through 7 following the initial wounding on day 0, five to eight wounds were removed from each group with a Castroviejo dermatome. The dermatome was equipped with a 22-mm blade and set at a depth of 0.5 mm. This enabled the surrounding nonwounded skin to be removed with the wound site.

The samples were then incubated in 2N sodium bromide at 37 °C for six to eight hours, enabling the epidermis to be separated from the dermis. The epidermis was then evaluated grossly for defects. The defects were visualized as holes in the separated epidermal sheet or as a lack of epidermal continuum in the area that contained the wound. The wound was considered healed if there were no defects in the epidermis and not healed if there was one or more defect (Fig 1). Occasionally an intact crust (scab) attached to the epidermis prevented absolute visual confirmation of epidermal integrity, especially in untreated control wounds. Earlier studies[9] demonstrated microscopically that the

Fig 1.—Separated epidermal specimens containing wound sites. A, Day 3: not healed. B, Day 4: not healed. C, Day 5: not healed with crust. D, Day 5: healed with crust. E, Day 5: healed, no crust. F, Day 6: healed.



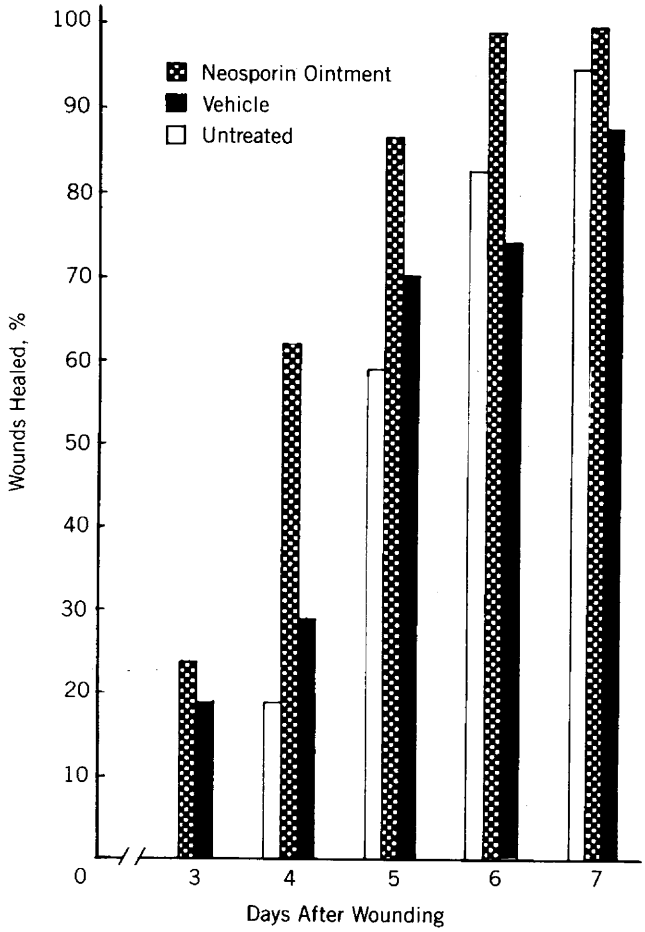


Fig 2.—Effect of Neosporin Ointment, vehicle, and control on wound healing.

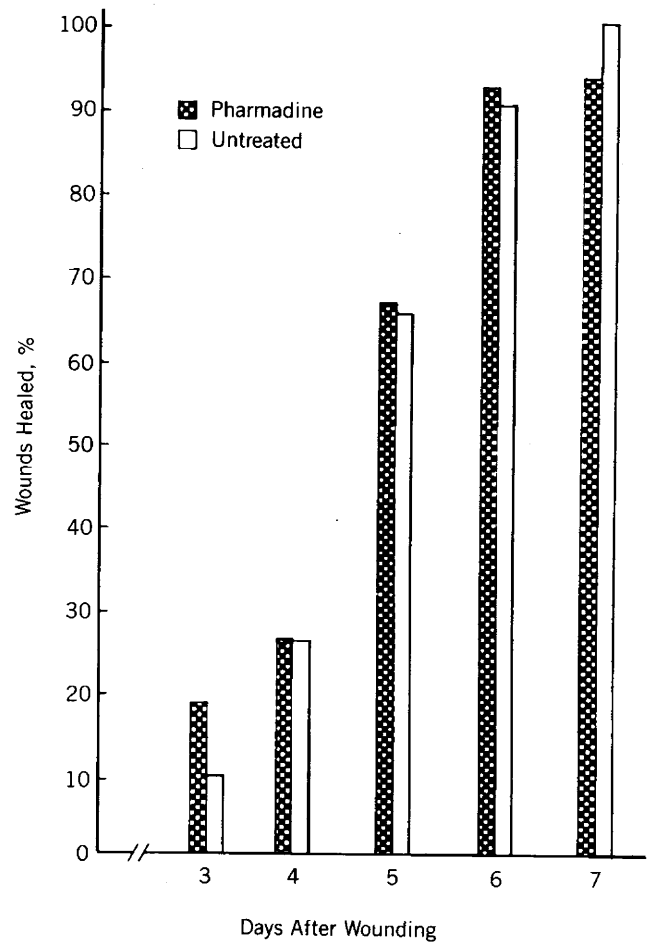


Fig 3.—Effect of Pharmadine and control on wound healing.

Table 1.—Effect of Agents and Vehicles on Healing*

Agent	Day 2	3	4	5	6	7
Neosporin group						
Untreated	0/17 (0)	0/27 (0)	5/27 (19)	19/32 (59)	25/30 (83)	20/21 (95)
Neosporin Ointment	0/19 (0)	5/21 (24)†	16/26 (62)‡§	26/30 (87)	26/26 (100)	19/19 (100)
Vehicle	0/15 (0)	5/27 (19)	8/28 (29)	21/30 (70)	20/27 (74)	22/25 (88)
Pharmadine group						
Untreated	...	2/20 (10)	5/19 (26)	17/26 (65)	18/20 (90)	13/13 (100)
Pharmadine	...	4/21 (19)	6/27 (27)	19/29 (66)	22/24 (92)	14/15 (93)
Furacin group						
Untreated	0/15 (0)	1/32 (3)	10/36 (28)	33/48 (69)	43/47 (91)	12/12 (100)
Furacin	0/14 (0)	0/21 (0)	1/27 (4)	2/34 (6)¶	17/32 (53)†	8/10 (80)
Vehicle	1/27 (4)	1/23 (4)	2/23 (9)	18/30 (60)	27/30 (90)	10/13 (77)
Silvadene group						
Untreated	0/59 (0)	4/71 (6)	35/86 (41)	44/77 (57)	...	...
Silvadene	0/15 (0)	14/29 (48)†	25/28 (89)¶	21/21 (100)	...	...
Vehicle	1/61 (2)	23/81 (28)†	78/93 (84)¶	83/90 (92)	...	...

*Values are expressed as number of specimens healed/total number of specimens tested. Numbers in parentheses are percent healed.
†$P < .05$ compared with untreated.
‡$P < .01$ compared with untreated.
§$P < .05$ compared with vehicle.
¶$P < .001$ compared with untreated.

Table 2.—Comparative Rates of Healing With Agents and Vehicles Tested

Agent	HT_{50}, Days*	Relative Rate of Healing Compared With Control, %
Neosporin group		
Untreated	4.8	0
Neosporin Ointment	3.6	+25
Vehicle	4.6	+5
Pharmadine group		
Untreated	4.6	0
Pharmadine	4.55	+1
Furacin group		
Untreated	4.6	0
Furacin	6.0	−24
Vehicle	4.8	−9
Silvadene group		
Untreated	4.3	0
Silvadene	3.1	+25
Vehicle	3.4	+21

*HT_{50} is the time needed for 50% of the wounds to heal.

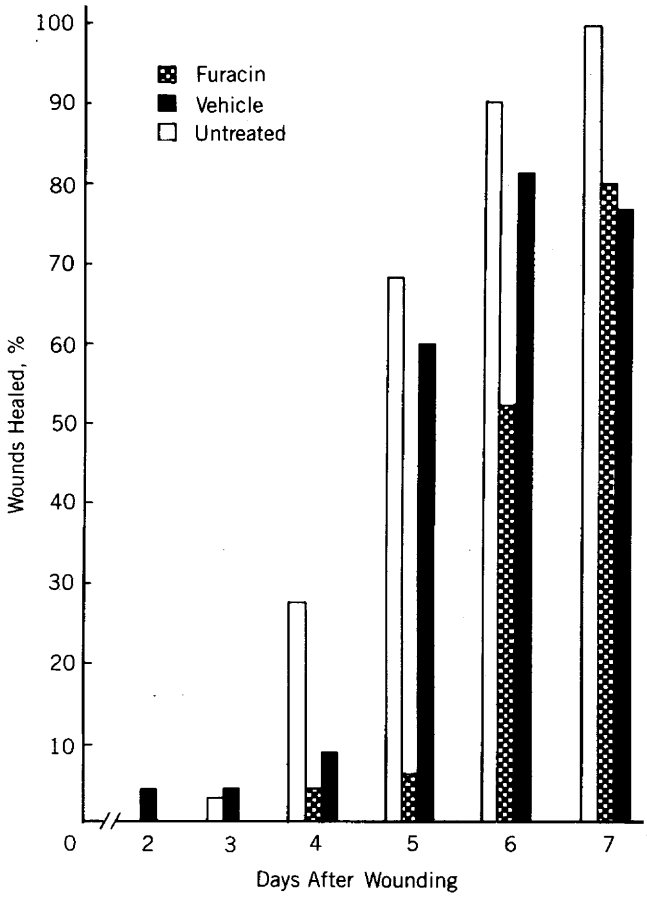


Fig 4.—Effect of Furacin, vehicle, and control on wound healing.

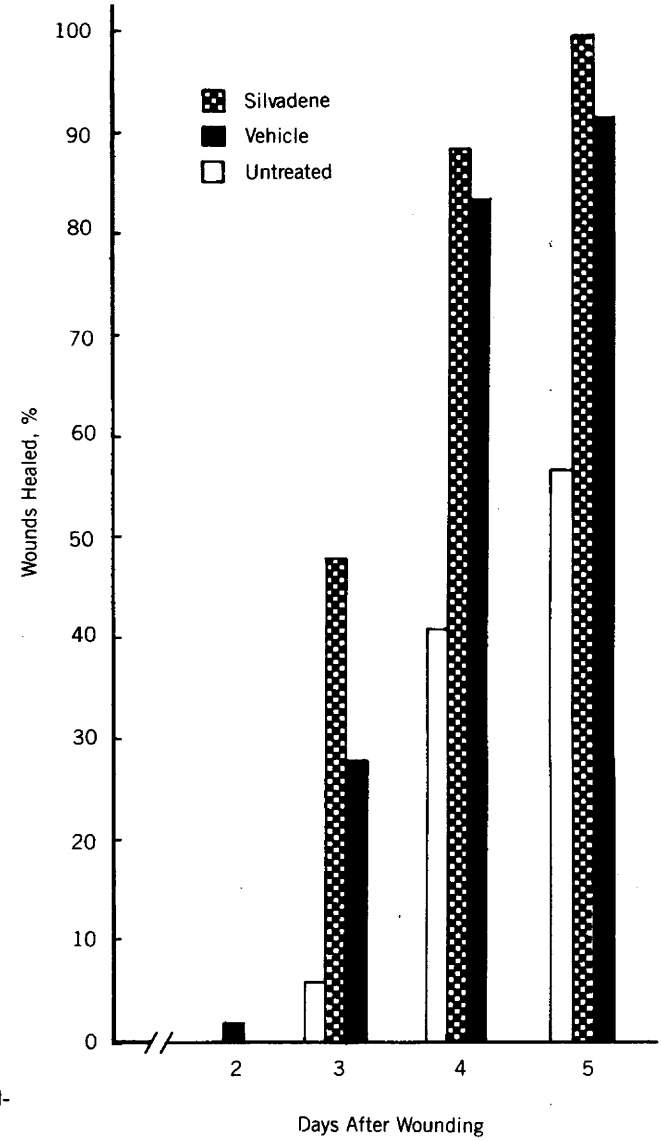


Fig 5.—Effect of Silvadene, vehicle, and control on wound healing.

crusts adhere to the dermal portion of the specimen and leave a defect unless epidermis is present beneath the crust.

Treatment

The wounds on each experimental animal were divided randomly into three treatment categories: (1) active agent, (2) vehicle of the active agent, and (3) untreated control. In the study of the povidone-iodine solution the vehicle was not tested. The wounds in each category were grouped together to avoid spread of one agent to another area. The location of the group of wounds was varied randomly in subsequent experimental animals. Approximately 0.1 mL of the topical agents was applied once daily, for six days, to the wound or crust.

RESULTS

The results of the treatment are summarized in Table 1 and Fig 2 to 5. The time needed for 50% of the wounds in each experimental animal to heal (HT_{50}) has been estimated from curves generated by probit analysis of the data presented in Table 1. The HT_{50} values are compared in Table 2.

COMMENT

The lack of data on the effects of topical antimicrobial agents on wound healing has led to numerous theories on the subject. It has been demonstrated that bacterial overgrowth and the presence of certain bacteria or their metabolites can cause an inhibition of wound healing.[12] This has led to claims by drug manufacturers and others[1] that prevention of bacterial growth can aid or promote the healing of wounds. Others have reported that there is no substantial evidence to believe that these agents can shorten healing time by reduction of the number of bacteria,[13] while a third opinion has been expressed by Faddis et al,[14] who demonstrated that some topical agents can cause tissue toxicity, which might lead to a delay in healing.

The rate of reepithelialization of a wound can be altered by changes in the wound's local environment[15-17] and by application of topical agents.[9,18] Even various vehicles that were previously thought to be "inert" have been shown to have measurable effects on wound healing.[19] Therefore, it is very likely that topical antimicrobial agents have the potential to affect the rate of healing through a number of possible mechanisms. These possibilities include the antimicrobial effect of these agents, which would alter the wound's environment and any other effect, whether it be biochemical, metabolic, or physical, caused by the vehicles of the active agents or by the active agents themselves.

Neosporin Ointment consists of

three antibiotics, neomycin sulfate, polymyxin B sulfate, and bacitracin zinc, in a petrolatum base. This agent covers a wide spectrum of antibacterial activity including most Gram-positive and Gram-negative bacteria found in human and pig skin. We found that the active agent promoted healing by 25% compared with the untreated control. This increase was significant compared with both the vehicle and the control. However, there was no significant difference noted between the vehicle and the control.

Petrolatum products differ widely in their preparation and physical properties.[20] The petrolatum vehicle of Neosporin Ointment has a lower melting point than *United States Pharmacopeia* petrolatum and differences in healing rates have been noted between the two. The petrolatum vehicle showed an insignificant increase in healing time compared with a significant slowdown of 17% reported previously with USP petrolatum.[19] We confirmed the difference in the effect of these two types of petrolatum on healing in studies directly comparing these two.

Pharmadine is a povidone-iodine solution with 9% to 12% available iodine. Its antimicrobial spectrum includes Gram-negative and Gram-positive bacteria. The povidone-iodine solutions have gained wide use as prophylactic treatment for minor procedures and in preoperative preparation. Despite the large overlap in antibacterial activity with Neosporin Ointment, we did not find an increased healing rate in Pharmadine-treated wounds as was found in those wounds treated with Neosporin Ointment.

Furacin contains nitrofurazone in Solubase, which is a water-soluble base of polyethelene glycols. This agent is used primarily as adjunct therapy in second-degree burns. As noted with Neosporin Ointment and Pharmadine, Furacin was the only topical antimicrobial agent we examined that demonstrated an inhibition of healing. The 24% slowdown was statistically significant compared with Solubase and its control.

Silvadene cream contains 1% sulfadiazine silver in a water-miscible cream. This product has been used extensively for the treatment of burns. Its antibacterial activity covers a wide sprectrum of Gram-positive and Gram-negative bacteria and also fungi. Silvadene promoted healing at the fastest rate of the agents in the study, being 28% faster than the control. Both the active agent and its base were significantly faster than the untreated control. There was no significant difference between the active agent and its vehicle.

Although we have demonstrated that topical antimicrobial agents can alter the rate of reepithelialization, their mechanisms of action are not clear, and probably differ from each other. From analysis of our data it appears that the effect on healing produced by these agents is not due to their antimicrobial action. The effect of Silvadene cream vehicle relative to the cream containing the active agent minimizes the possibility that this product influences wound healing by its antibacterial effect. Since Neosporin Ointment's vehicle had no real effect on healing and Pharmadine's vehicle consists mostly of distilled water, it would seem reasonable that the two products would influence wound healing similarly because of the similarity in the antibacterial spectrums of their active agents. However, this is not the case (Tables 1 and 2). Since povidone-iodine solutions have little tissue toxicity[14] and proven antibacterial action, the possibility of wounds not healing rapidly with Pharmadine treatment because of tissue toxicity or insufficient antibacterial activity is remote.

Although Furacin has an antibacterial spectrum comparable to two agents that we have found to promote wound healing, it was found to significantly retard the rate of reepithelialization. The mechanism of its antimicrobial action differs from other agents in this study in that it inhibits enzymes that are necessary for carbohydrate metabolism in both the aerobic and anaerobic cycles of bacteria. The *Physician's Desk Reference*[2] states that Furacin is without appreciable toxicity to human cells; however, we know of no data to support this statement. The possibility that Furacin retards reepithelialization by virtue of its toxicity to epidermal cells cannot be excluded.

To evaluate further the idea that these agents might influence wound healing by their antimicrobial action, we ruled out the possibility, in an unpublished study (W. H. Eaglstein, MD, P. Mertz, October 1978), that the results were related to changes in the number or types of bacteria within the wound.

Since we have shown that topical antimicrobial agents and their vehicles can either promote or retard the rate of reepithelialization of a wound, most likely through a mechanism other than their antimicrobial action, it would appear worthwhile to evaluate the remainder of the commonly used topical agents that were not examined in this study. An understanding of these agents' mechanisms of action could conceivably lead to the development of new agents that might promote healing, while identifying those agents and factors that retard the healing process.

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