
Irritation and allergy patch test analysis of topical treatments commonly used in wound care: Evaluation on normal and compromised skin

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Background: Topical agents indicated for the treatment of superficial wounds have the potential to cause irritation or allergic contact dermatitis, particularly when applied to an impaired skin barrier.

Objective: We sought to compare the irritancy potential of 5 topical wound care products commonly used in dermatologic practice on normal and compromised skin.

Methods: Agents tested included Aquaphor Healing Ointment (AHO) (Beiersdorf Inc, Wilton, CT); bacitracin; Biafine Topical Emulsion (BTE) (OrthoNeutrogena, Los Angeles, CA); Neosporin (Poly/Bac/Neo) (Johnson & Johnson, New Brunswick, NJ); and Polysporin (Poly/Bac) (Johnson & Johnson). Study 1 assessed cumulative irritation using a modified human repeat insult patch test on normal back skin with an induction phase (test materials applied under occlusive patch 9 times at 48- to 72-hour intervals) and a challenge phase (test materials applied to original and naïve sites for 48 hours, 12-24 days postinduction). Irritation was graded for erythema and type IV allergy skin responses. Study 2 assessed the acute irritation potential of agents on tape-stripped (“wounded”) back skin. Test sites were graded for erythema, transepidermal water loss, and skin color (Chroma Meter a*) (Minolta, Osaka, Japan) at 48 and 72 hours poststripping.

Results: In study 1, cumulative irritation testing in 108 subjects classified AHO, bacitracin, Poly/Bac/Neo, and Poly/Bac as “mild,” and BTE as “probably mild.” In study 2 at 72 hours, mean clinical grading scores were significantly higher for BTE and Poly/Bac/Neo than AHO. Transepidermal water loss and colorimeter a* values were significantly lower for AHO and bacitracin compared with BTE. No allergic contact dermatitis was seen in either study.

Conclusions: Patch test studies demonstrated that BTE showed the greatest irritancy potential in both normal and compromised skin whereas AHO showed the least. (J Am Acad Dermatol 2011;64:S16-22.)

Key words: allergic contact dermatitis; Aquaphor Healing Ointment; bacitracin; Biafine Topical Emulsion; cumulative irritation; Neosporin; patch testing; Polysporin.

Over-the-counter antibiotic-based ointments are frequently used to treat skin wounds resulting from minor surgical procedures.¹

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Abbreviations used:

ACD:	allergic contact dermatitis
AHO:	Aquaphor Healing Ointment
BTE:	Biafine Topical Emulsion
NACDG:	North American Contact Dermatitis Group
Poly/Bac:	Polysporin
Poly/Bac/Neo:	Neosporin
TEWL:	transepidermal water loss

There is evidence, however, that these products may cause allergic contact dermatitis (ACD) in a portion of the population. The North American Contact Dermatitis Group (NACDG) has reported neomycin allergies in 7% to 13% of patch-tested patients over the last 20 years.² A study of postoperative use of

neomycin on minor surgical wounds reported ACD in 5.3% of patients.³ Neomycin was named "Contact Allergen of the Year" in 2010 by the NACDG.⁴ The NACDG has also reported an increasing incidence of bacitracin allergies, from 1.5% between 1985 and 1990 up to 7.7% to 9.2% between 2000 and 2004,² resulting in it naming bacitracin "Contact Allergen of the Year" in 2003.⁵

Although less dramatic than the immune response seen in ACD, repeated exposure to an irritant can result in a cumulative irritation reaction, which is localized to the site of contact.^{6,7} Irritants, particularly small molecules such as antibiotics, have the potential to cause sensitization and subsequent allergic responses upon repeated exposure, resulting in edema and a spreading skin reaction beyond the site of exposure.^{7,8} Prolonged use of topical antibiotics, coupled with an impaired skin barrier, increases the risk of developing ACD as a result of the increased availability of the potential allergen. Several studies have found that ACD is more common in patients with postoperative or posttraumatic wounds, chronic venous insufficiency, chronic otitis externa, and chronic eczematous conditions.²

The treatment of minor wounds with topical antibiotics is commonplace, as it is believed that they are necessary to reduce the risk of infection. However, studies have shown comparable healing rates between white petrolatum and the antibiotics bacitracin⁹ and gentamicin¹⁰ with similar rates of infection, and there is little evidence that topical antibiotic use has decreased infection rates.¹¹ In contrast, there is considerable evidence that topical antibiotics can cause ACD.^{3,9,10,12} In addition, several topical antibiotics have been shown to have cytotoxic effects on human fibroblasts and keratinocytes at clinically relevant doses.¹³ Polysporin (Poly/Bac) (polymyxin B sulfate/bacitracin zinc) (Johnson & Johnson, New Brunswick, NJ), bacitracin, and polymyxin B have all been shown to significantly decrease cell proliferation of fibroblasts and reduce the number of viable keratinocytes during *in vitro* studies when administered at clinical doses.¹³ Animal model studies have shown that this disruption of fibroblasts by topical antibiotics impedes wound contraction, part of the normal process of wound healing.¹⁴

Diagnosis of ACD requires careful assessment by a dermatologist. Patch testing is the standard tool for diagnosing and confirming the possibility of suspected allergies.² In addition, patch-testing techniques are routinely performed in industry to evaluate the cumulative irritancy and ACD potential of topical formulations and to support the product safety dossier. Here, we report on two blinded patch-testing studies performed to evaluate

predictive allergy and irritancy potential of 5 topical wound care products commonly used in dermatologic practice. Three of these products were antibiotic-based ointments: bacitracin; the combination antibiotic ointment Neosporin (Poly/Bac/Neo) (neomycin, polymyxin B sulfate, and bacitracin zinc) (Johnson & Johnson); and Poly/Bac. Poly/Bac and Poly/Bac/Neo are the two most commonly used wound care treatments in dermatology offices. The other two products were antibiotic-free formulations: the skin protectant Aquaphor Healing Ointment (AHO) (Beiersdorf Inc, Wilton, CT) and the medical device Biafine Topical Emulsion (BTE) (OrthoNeutrogena, Los Angeles, CA). All of these agents are indicated for the treatment of superficial wounds and minor abrasions. BTE, the sole prescription product tested, is also indicated for the treatment of dermal ulcers, donor sites, first-degree and second-degree burns, and radiation dermatitis.¹⁵ Study 1 tested these products for the combined cumulative irritation and the potential to induce allergy on normal back skin using patch-testing techniques. Study 2 was an exaggerated test involving tape-stripped skin designed to assess the acute irritation potential of these products on wounded or compromised skin. Back skin was tape-stripped to mimic the conditions of a minor wound. Test products were applied to each tape-stripped site under an occlusive patch to assess irritation potential on "wounded" skin.

METHODS

Study 1: Modified human repeat insult patch test to evaluate cumulative irritation and predictive allergy

Men and women aged 18 to 70 years with Fitzpatrick skin type I to IV were enrolled in the study. Individuals were excluded if they had any active or history of skin diseases or conditions that may interfere with the evaluation of skin reactions, had any known allergy to skin care products, or routinely received any anti-inflammatory medications. All participants were required to sign an informed consent agreement consistent with the requirements of 21 Code of Federal Regulations 50.25.

This study used a modified human repeat insult patch test developed by Rizer and Nozawa¹⁶ that combines cumulative irritation assessment in the induction phase and potential for induction of allergy during the challenge phase. The test protocol is routinely used in Japan and North America for developing product safety dossiers. The test design includes an induction phase and a challenge phase. During the induction phase the test products are applied under occlusive patch 9 times over 3 weeks.

Table I. Irritation grading scale for exaggerated irritation patch study

Grade	Description
0.0	No apparent cutaneous involvement
0.5	Faint, barely perceptible erythema or slight dryness (glazed appearance)
1.0	Faint but definite erythema, no eruptions or broken skin, or no erythema but definite dryness; may have epidermal fissuring
1.5	Well-defined erythema or faint erythema with definite dryness; may have epidermal fissuring
2.0	Moderate erythema; may have few papules or deep fissures, moderate to severe erythema in cracks
2.5	Moderate erythema with barely perceptible edema or severe erythema not involving significant portion of patch (halo effect around edges); may have few papules or moderate to severe erythema
3.0	Severe erythema (beet redness); may have generalized papules or moderate to severe erythema with slight edema (edges well defined by raising)
3.5	Moderate to severe erythema with moderate edema (confined to patch area) or moderate to severe erythema with isolated eschar formations or vesicles
4.0	Generalized vesicles or eschar formations or moderate to severe erythema and/or edema extending beyond area of patch

Patch sites are graded using the Berger and Bowman¹⁷ skin irritation grading scale. From these data, cumulative irritation potential is determined based on a standardized interpretation system established by Berger and Bowman.¹⁷ The challenge phase occurs between 12 and 24 days after the final induction phase patch. The grading scale includes macular and indurated erythema and elevated skin responses characteristic of type IV allergy (papules, vesicles, bullae, spreading, and weeping).¹⁸

Test products for this analysis included AHO, Poly/Bac, Poly/Bac/Neo, bacitracin, and BTE. AHO is composed of petrolatum (41%), mineral oil, cerasin, lanolin alcohol, panthenol, glycerin, and bisabolol. Poly/Bac/Neo ointment contains polymyxin B sulfate (5000 U/g), bacitracin zinc (400 U/g), and neomycin (3.5 mg/g) in a vehicle made up of cocoa butter, cottonseed oil, olive oil, sodium pyruvate, tocopheryl acetate, and white petrolatum. Poly/Bac ointment contains bacitracin zinc (500 U/g) and polymyxin B sulfate (10,000 U/g) in a white petrolatum vehicle. BTE is composed of purified water, liquid paraffin, ethylene glycol monostearate, stearic acid, propylene glycol, paraffin wax, squalane oil, avocado oil, trolamine/sodium alginate, triethanolamine, cetyl palmitate, methylparaben (sodium salt), sorbic acid (potassium salt), propylparaben (sodium salt), and fragrance.¹⁹ A positive control (1% sodium lauryl sulfate) and a negative control (undosed patch) were included.

Study 2: 48-hour exaggerated irritation patch study

This double-blind randomized study involved a barrier disruption of the skin, followed by occlusive patch application of the test materials AHO, Poly/Bac, Poly/Bac/Neo, bacitracin, and BTE.

Inclusion and exclusion criteria were the same as in study 1. Each 1- × 1-inch test site was tape-stripped to the glistening layer to mimic a wound. Transepidermal water loss (TEWL) was measured after tape-stripping using DermaLab (Cortex Technologies, Hadsund, Denmark) to monitor barrier disruption. Test products were applied to the tape-stripped sites under occlusive patch for 48 hours. Test sites were graded for irritation at 48 hours and 72 hours after tape-stripping/patching using the primary irritation grading scale described in Table I.

In addition, TEWL and skin color were measured using DermaLab (Cortex Technologies) and Chroma Meter CR-400 (Minolta, Osaka, Japan), respectively. TEWL is a measurement of the rate of water loss from the skin in g/m²/h and is an indicator of skin barrier function. The Chroma Meter (Minolta) colorimeter measures skin luminosity (L*) and erythema (a*), using silicone photocells calibrated to match the Commission Internationale de l'Éclairage standard 3-dimensional color space L*, a*, and b*.²⁰

Clinical grading results were compared between test products using analysis of variance with pairwise comparisons (Fisher least squares difference). The TEWL and colorimeter measurements taken at 48 and 72 hours after tape-stripping/patching were compared between test products, also using analysis of variance with pairwise comparisons (Fisher least squares difference).

RESULTS

Study 1: Modified human repeat insult patch test to evaluate cumulative irritation and predictive allergy

Of the 130 subjects who enrolled in this study, 108 subjects completed, with 21 withdrawals for reasons

Table II. Demographics

	Study 1	Study 2
	Cumulative irritation study on normal skin N = 108	Irritation study on compromised skin N = 16
Gender, n (%)		
Male	18 (16.7)	2 (12.5)
Female	90 (83.3)	14 (87.5)
Age range, y (mean)	18.2-65.1 (44.2)	20.7-65.4 (46.4)
Ethnicity, n (%)		
Caucasian	79 (73.2)	16 (100)*
Hispanic	17 (15.7)	
Native American	5 (4.6)	
African American	4 (3.7)	
Other (mixed)	3 (2.8)	

*Fitzpatrick skin type: 25% type I (n = 4); 75% type II (n = 12).

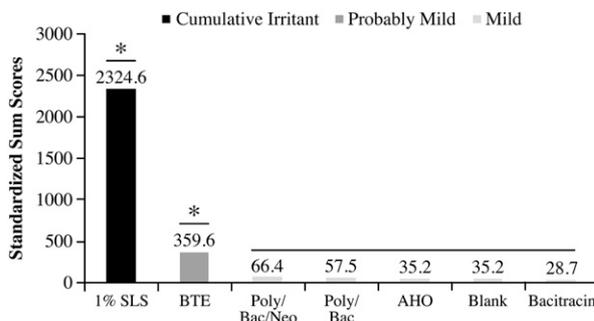
unrelated to the study. The demographics of the patient population are shown in Table II.

The cumulative irritation potential of AHO, bacitracin, Poly/Bac/Neo, and Poly/Bac during the induction phase was classified according to the Berger and Bowman¹⁷ standardized interpretation system as “mild material with no experimental irritation,” as was the undosed control patch (Fig 1). BTE was classified as “probably mild in normal use.” The 1% sodium lauryl sulfate positive control was classified as a “cumulative irritant.” During the induction phase, the incidence of irritation scores of 2 or 3 was nonexistent for AHO; minimal for bacitracin, Poly/Bac, and Poly/Bac/Neo; and marked (23.1%) in the test population for BTE (Fig 2). No scores greater than 3 were recorded for any of the products. The challenge phase grading of the original and naïve sites showed no evidence of induced ACD for any of the test materials.

Study 2: 48-hour exaggerated irritation patch study

Sixteen subjects meeting the inclusion and exclusion criteria were enrolled and completed the study (Table II). The mean clinical grading scores for the exaggerated irritation patch sites showed no difference between the test materials or the negative control site after 48 hours (Fig 3). After 72 hours, BTE and Poly/Bac/Neo showed significantly higher erythema scores compared with the negative control and AHO.

For the instrumental analyses, the colorimeter a* values significantly increased relative to the baseline for all test materials and the negative control at 48 and 72 hours after tape-stripping (Fig 4). No significant differences in a* values between any of the



*P≤0.05

Fig 1. Cumulative irritation scores for 5 test materials and controls after 9 consecutive patches. Sum scores are shown. Standardized sum scores were used to classify test materials as mild, probably mild, and cumulative irritant, according to Berger and Bowman¹⁷ classification. Blank is negative control and 1% SLS (sodium lauryl sulfate) is positive control. *P ≤ .05 for comparison between irritation classifications. Items marked by same line are not significantly different from one another.

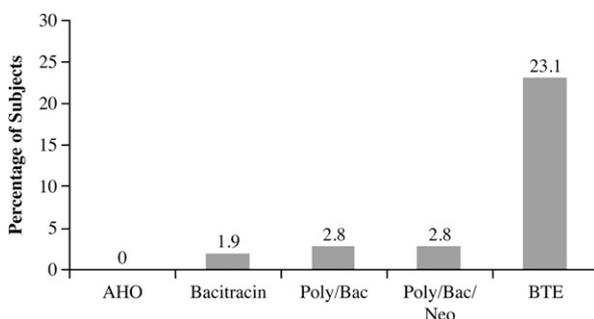


Fig 2. Incidence of clinical grading scores of 2 or 3 (N = 108). Scores were based on Berger and Bowman¹⁷ skin irritation grading scale. Grading of irritation occurred during induction phase of modified human repeat insult patch test.

patched sites were observed at 48 hours; however, at 72 hours, significantly less redness was observed at the AHO- and bacitracin-treated patch sites compared with BTE; the a* values for the sites treated with AHO and bacitracin were equivalent to the negative patch site (not shown). TEWL at 48 hours showed that the sites patched with bacitracin and AHO and the negative patch site (not shown) were statistically equivalent and had significantly less water loss than those patched with BTE (Fig 5). All treatment sites were statistically equivalent after 72 hours.

DISCUSSION

No evidence of induced ACD was seen with any of the test products. BTE caused the most cumulative irritation of all of the test materials, whereas AHO

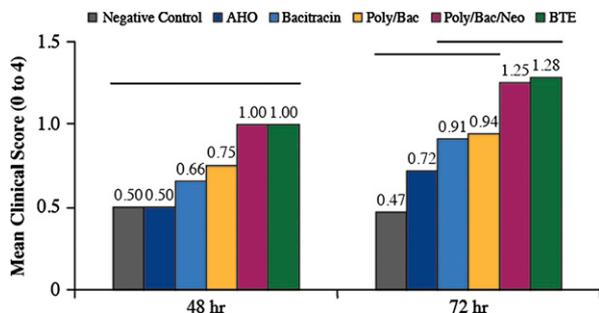


Fig 3. Acute irritation on tape-stripped skin: mean clinical grading scores at 48 and 72 hours. Mean clinical scores were graded from 0 to 4. Scale is shown for 0 to 1.5 for clarity. Items marked by same line are not significantly different from one another.

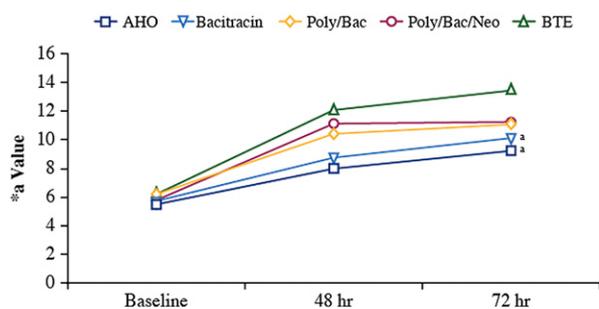


Fig 4. Acute irritation on tape-stripped skin: colorimeter a* (redness) values. ^aP value significantly different from BTE. ($P \leq .05$).

treatment resulted in the least irritation. Poly/Bac, Poly/Bac/Neo, and bacitracin were shown to have similar irritation potential on normal skin; however, on tape-stripped “wounded” skin, the triple antibiotic ointment Poly/Bac/Neo appeared to cause the most irritation of the antibiotic-based ointments.

Study 1 used a modified human repeat insult patch test design that allows the assessment of both cumulative irritation and ACD.¹⁶ The results from study 1 showed that all treatments had a Berger and Bowman¹⁷ cumulative irritation classification of “mild in normal use,” with the exception of BTE, whose cumulative irritation was significantly greater than other treatments and classified as “probably mild in normal use.” Although patch testing under occlusive conditions is an exaggerated use situation, in the case of evaluating wound care products, occlusive patching conditions may emulate common practice; such products are often applied daily (or even more frequently) to wounds and are generally kept covered with a bandage for at least the early stages of healing. Although it is not possible to identify the exact cause of the higher irritancy seen with BTE, this product contains propylene glycol,²¹

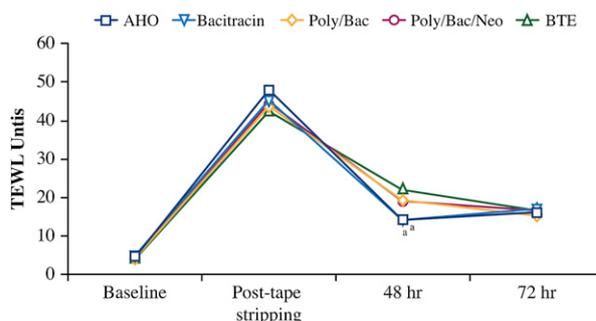


Fig 5. Transepidermal water loss (TEWL) for exaggerated patch test study: measure of barrier function. Instrumental analysis of water loss because of damage of stratum corneum in $\text{g}/\text{m}^2/\text{h}$. ^aP value significantly different from BTE. ($P \leq .05$).

paraben-based preservatives,²² and fragrance,²³ which all have the potential to cause irritation (see “Methods” section for complete list of ingredients). In addition, BTE had the highest incidence of erythema during the induction phase. In contrast, AHO exhibited no cumulative irritation grading scores of 2 or greater, whereas bacitracin, Poly/Bac/Neo, and Poly/Bac had low rates.

The results from the challenge phase demonstrated that none of the treatments induced an allergic response in this study population. This was not unexpected. The incidence of ACD for the tested antibiotics is reported as ranging from 5% and 13%.^{2,3} The sample size ($N = 108$) may appear to have been large enough for ACD to have been detected; however, two factors may explain why it was not observed in this study. First, in the recruitment process, individuals with known allergy to first-aid ointments or creams were excluded from participation. Second, the prevalence of ACD in the general population is unknown.² Reported rates of ACD are typically derived from data collected from patch-tested individuals who were tested because of suspicions of sensitization, therefore representing a selected population.

Study 2 used a 48-hour occlusive patch on tape-stripped skin. The skin was tape-stripped down to the glistening layer to mimic a superficial wound and to increase the sensitivity of the method to assess irritation. Once the stratum corneum is removed, the barrier function is compromised and the skin is more sensitive to irritants. Patch testing tape-stripped skin mimics the practice of applying topical ointments and a bandage after a wounding procedure and determines if any of the test materials cause acute objective irritation.

The clinical grading scores showed no significant differences in erythema between any of the products

after 48 hours. At 72 hours, however, Poly/Bac/Neo and BTE had significantly higher erythema scores than the AHO and negative control site. Although there were significant differences in erythema, it should be noted that the mean erythema grading scores for all of these products was relatively low.

Objective instrumental measurements were used in this study to complement the clinical observations.^{20,24} Chroma Meter (Minolta) technology is widely used in dermatology research and is an effective tool for quantifying erythema. The a* value measures skin color hues from red to green, with increasing scores indicating greater vascularization or blood flow, ie, erythema. In the exaggerated patch test on compromised skin, the a* values showed an increase in erythema at 48 hours after tape-stripping for all sites, as expected. At 72 hours, sites treated with BTE showed the most erythema, whereas sites treated with AHO and bacitracin showed the least; however, all sites showed increased erythema at 72 hours from 48 hours, which is a normal consequence of the wound healing process. These findings parallel the mean erythema clinical grading scores; at 72 hours, both clinical grading and colorimeter erythema assessments were able to differentiate the irritant effects of some of the test products.

TEWL measurements confirmed that the barrier was disrupted after tape-stripping and the extent of repair differed among the various products. The 48-hour TEWL measurements showed some significant differences in skin barrier integrity between sites treated with the different products. AHO and bacitracin showed significantly improved barrier recovery relative to the other products. After 72 hours, TEWL was similar for all sites, indicating the barrier had recovered to the same degree for all treated sites.

Overall, the clinical and instrumentation analyses confirmed that AHO was the least irritating of all the products, whereas BTE was the most likely to cause irritation. The 3 antibiotic-based ointments were comparable for most parameters, with bacitracin probably the least irritating of the 3. This is not surprising as all 3 ointments contain bacitracin. Poly/Bac/Neo also contains neomycin, which has been documented to cause ACD so frequently that it is part of the standard series of patch tests.^{1,25}

These studies confirm that AHO is a mild skin protectant ointment that causes less irritation than 3 commonly used topical antibiotic-based ointments and the topical emollient, BTE. Under the conditions of these studies, AHO was shown to have the lowest irritation profile on normal and compromised skin.

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