In Vivo Method for Determining Effectiveness of Spray-on Bandages Containing Anti-Infectives

M. LUONGO, J. J. SCIARRA ˣ, and C. O. WARD

Abstract □ Several topical spray-on bandages were developed and studied to determine a suitable method for evaluating the effectiveness of this group of products. Several formulations were prepared using neomycin sulfate and triclosan. Ethylcellulose and polyamide resins served as the film-forming agents, while tributyl citrate and hexadecyl alcohol were chosen as the plasticizers. Neomycin sulfate and triclosan preparations were evaluated using an *in vivo* method. Standardized, contaminated wounds in guinea pigs served as the testing system. The inflammatory response of the wounds was evaluated using several indexes including skin temperature, degree of induration, and presence of pus. The results indicate that neomycin sulfate and triclosan were released from their formulations. The spray-on bandages reduced the degree of infection about the wound. No difference was noted between the results obtained using either film former.

Keyphrases □ Spray-on bandages containing anti-infectives (neomycin sulfate and triclosan)—determination of *in vivo* effectiveness, guinea pigs □ Anti-infective spray-on bandages (neomycin sulfate and triclosan)—determination of *in vivo* effectiveness, guinea pigs □ Aerosol anti-infectives (neomycin sulfate and triclosan)—determination of *in vivo* effectiveness of spray-on bandages, guinea pigs

In recent years, great interest has developed regarding the use of medicated polymeric films in managing contaminated wounds. The value of using anti-infectives to control infections also has been firmly established over the years. On this basis, it seemed advantageous to formulate a preparation in which an anti-infective compound is released from a polymeric film following application onto the skin. A spray-on bandage formulated in this manner would have many advantages including ease of application, prevention of contamination of the product by the user, and, depending upon the nature of the polymeric film and therapeutic agent, a slow release of the active compound.

Spray-on bandages using polyvinyl alcohol in combination with polyvinyl acetate or acrylic resins and other water-soluble resins were investigated (1), and butyl cyanoacrylate was shown to be an effective chemical compound (2). In one study (3), optimal therapeutic responses were seen in the contaminated soft tissue wounds in rats when an antibiotic was administered to the affected area and covered with a spray dressing of bucrylate (isobutyl cyanoacrylate). Chloramphenicol and nitrofurazone were incorporated into solutions of ethylcellulose and sodium carboxymethylcellulose (4).

The possibility of infections developing after elective surgical procedures has been a recurring problem for both the physician and the patient. An antibiotic triad consisting of polymyxin, bacitracin, and neomycin was developed in the form of an aerosol dressing using fluorinated hydrocarbons as the propellant (5). The value of using aerosol sprays of oxytetracycline and a neomycin sulfate–polymyxin B sulfate product was demonstrated (6). Further work also was done in regard to using an antibiotic aerosol in minor surgery (7). Previous studies (8–10) indicated the use of certain polymeric films for formulating spray-on bandages and showed the release rate of different therapeutic agents from these films.

The purpose of this study was to select the proper combination of film former, plasticizer, and solvent to yield a film that permits drug release. Once the formulation is developed, an *in vivo* study will be conducted to determine the suitability of the method used as well as the relative effectiveness of the formulations.

Table I—Composition of Aerosols

Ingredient, % w/w	Formulation Number 5	5A	23	25
Polyamide resin[a]	—	2.5	—	25
Ethylcellulose	2.5	—	2.5	—
Neomycin sulfate	0.175	0.175	—	—
Triclosan	—	—	1.0	1.0
Tributyl citrate	0.25	0.25	0.25	0.25
Hexadecyl alcohol	0.25	0.25	0.25	0.25
Absolute ethanol	11.825	—	11.0	—
Absolute isopropyl alcohol	—	11.825	—	11.0
Propellant [trichloromonofluoromethane–dichlorodifluoromethane (50:50)]	85	85	85	85

[a] Emerez 1538.

EXPERIMENTAL

The polymers used included ethylcellulose[1] and two polyamide resins[2]. Hexadecyl alcohol and tributyl citrate[3] were used as the plasticizers. The therapeutic agents used included neomycin sulfate and triclosan[4].

Formulation—Formulations were prepared on a weight percent basis using varying combinations and amounts of the film-forming resins, plasticizers, solvents, and drugs. Absolute ethanol served as the solvent system for preparations containing ethylcellulose, while absolute isopropyl alcohol was used as the solvent for the polyamide resins. Heat was used to aid in the dissolution of the film formers.

The solution of film former, plasticizer, and solvent was added to a 202 × 214 internally lined, three-piece, tin-plated aerosol container and then fitted with an aerosol valve[5] and sealed. A propellant mixture of trichloromonofluoromethane and dichlorodifluoromethane (50:50) was added by the pressure method. The container was placed into a water bath at 56° to ensure that no leakage took place. A quick drying film was obtained when the concentrate was 15% (w/w) and the propellant was 85% (w/w) of the total

[1] Ethylcellulose N-10, Hercules Powder Co., Wilmington, Del.
[2] Emerez 1533, 1538, Emery Industries, Cincinnati, Ohio.
[3] Citroflex-4, Chas. Pfizer and Co., New York, N.Y.
[4] Irgasan DP-300, Ciba-Geigy Corp., Ardsley, N.Y.
[5] Precision Valve Corp.; 0.04 × 0.03-cm (0.018 × 0.013-in.) stem with a 0.2-cm (0.80-in.) housing; 0.016 mechanical breakup actuator.

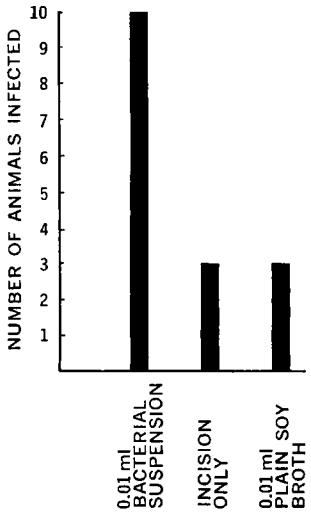

Figure 1—*Bar graph showing* in vivo *testing using controls.*

formulation. Various aerosol formulations were evaluated on the basis of film appearance and flexibility. These were then used for the *in vivo* test procedure. Table I shows the composition of the formulations selected for further study. These formulations gave satisfactory drying characteristics and films.

Delivery Rate—The delivery rate was determined using a suitable apparatus (11) which timed the product discharge. Each container was accurately weighed and then placed into a water bath at 25 ± 1°. After allowing the contents to come to equilibrium (about 1 hr), the container was removed from the water bath, dried, and placed into the discharge rate apparatus. The timer was set for 10 sec and the product was then allowed to be automatically discharged for this period of time. The can was removed from the apparatus and reweighed. Three determinations were made on each container. The discharge rate was determined by calculating the grams per second of product discharged. These results are shown in Table II and include the average of three determinations made on each of two cans of the same formulation.

***In Vivo* Study**—Adult male albino Hartley guinea pigs, weighing between 250 and 350 g, were used. Several hours before testing, the hair from the animal's back was clipped using an electric clipper and then a depilatory[6] was applied. The area was then washed with 70% ethanol. The skin temperature of the guinea pigs was taken at the star' of the test using a temperature probe. The animals were then anesthetized with sodium thiopental, 55 mg/kg body weight, administered intraperitoneally.

One group of animals received two incisions per animal, with each incision being treated with a different formulation; another group received only one incision per animal. Each incision was 3 cm long and parallel to and equidistant from the vertebral column. The incision was made through the skin down to the fascia using a scalpel blade fitted with a guide so that the skin would be cut to the proper depth.

Preparation of Bacterial Culture—*Staphylococcus aureus* (ATCC 12600) was obtained in the form of stable, standardized disks[7]. Each disk is standardized to contain 10^5–10^7 organisms when reconstituted in the proper culture medium. The disk was aseptically removed from the vial and placed into a capped sterile test tube containing 2 ml of sterile trypticase soy broth[8]. The tube was incubated at 35–37° for 30 min. It was then shaken and incubated at this temperature for an additional 4 hr.

Treatment of Animals—Once the bacterial suspension was prepared, the wounds were inoculated with 0.01 ml of the suspension delivered from a micropipet. This amount of suspension would contain approximately 5000–50,000 organisms.

The aerosol formulation was sprayed onto the wounded surface using a back-and-forth motion to cover the entire incision uniformly. The aerosols were sprayed onto the area for 5 sec at a distance of 15.2–20.3 cm (6–8 in.) from the wound surface. The aerosol film served as the only wound dressing. Where two incisions were made per animal, one incision was covered with gauze bandage while the therapeutic agent was being added to the other incision.

[6] Calcium thioglycolate, Nair-Carter Products, N.Y.

[7] Bact-Chek bacterial control cultures, Roche Diagnostics, Division of Hoffmann-La Roche, Inc., Nutley, N.J.

[8] BBL, Division of Becton, Dickinson and Co., Cockeysville, Md.

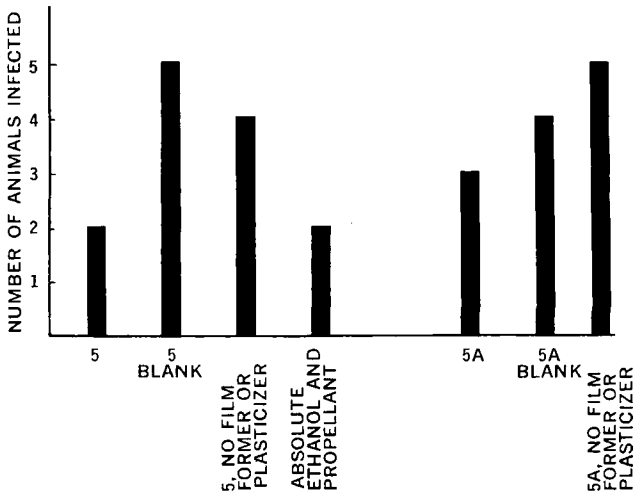

Figure 2—*Comparison of the results obtained from the* in vivo *testing of neomycin sulfate aerosol formulations. Key: 5, neomycin sulfate with ethylcellulose; and 5A, neomycin sulfate with polyamide resin.*

When neomycin sulfate ointment was used, approximately 1 g of the ointment was applied to the wound after it had been inoculated with the bacterial suspension. The wound was then covered with a sterile gauze pad and fastened with micropore tape. This ointment contained 3.5 mg of neomycin base. Neomycin sulfate ointment USP[9] was used in addition to the ointment prepared in the laboratory.

To determine the effect of the inactive ingredients contained in each aerosol preparation, blanks were also prepared containing no active ingredient, film former, or plasticizer. In addition, another blank consisted of alcohol and propellant alone.

Three control groups were included in this study. One group was inoculated with 0.01 ml of bacterial suspension only with no further treatment; another group received the 3-cm incision only, and the last group was treated by inoculating the wound with 0.01 ml of plain trypticase soy broth.

Evaluation of Wound Response—The inflammation of the infected wounds was evaluated in several ways including the deter-

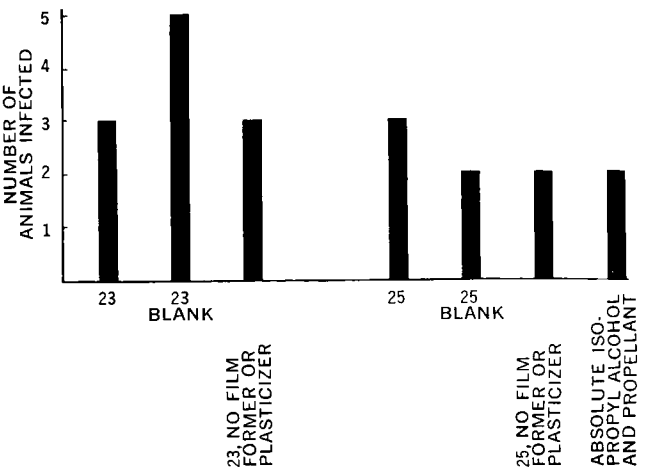

Figure 3—*Comparison of the results obtained from the* in vivo *testing of triclosan aerosol formulations. Key: 23, triclosan with ethylcellulose; and 25, triclosan with polyamide resin.*

[9] Myciguent Ointment, The Upjohn Co., Kalamazoo, Mich.

Table II—Delivery Rate and Active Ingredient Weight of Aerosols Selected for Animal Testing

Formula Number	Delivery Rate, g/sec	Active Ingredient, % in Film	Weight of Total Product/5 sec Spray	Weight of Non-volatile Film Remaining, g	Active Ingredient in Remaining Film, mg
5	0.551	5.512	2.755	0.08748	4.82
5A	0.673	5.512	3.365	0.10684	5.89
23	0.487	25.0	2.435	0.0974	24.35
25	0.474	25.0	2.370	0.0948	23.70

mination of skin temperature of the wounded area by observation of the color change of a thermographic liquid crystal tape[10] applied to the animal's back, induration of wound edges, and the presence or absence of pus. The animals were examined for these responses on the 4th postoperative day.

The skin temperature of the incision area was measured with a thermometer[11]. The liquid crystal tape was applied to the animal's back and allowed to remain there until the color stabilized. The area of induration was determined by palpating the margin of each wound and then measuring the indurated area in millimeters. After these responses were obtained, the wound was opened to detect any evidence of pus. The degree of infection that resulted was classified into one of four categories:

uninfected	no induration
minimal	1 mm induration or less
moderate	1.1–1.5 mm induration
massive	1.6 mm or more induration or presence of pus

The degree of infection caused by each control is shown in Fig. 1. Figure 2 illustrates the results using the formulation containing neomycin, and Fig. 3 shows these results using triclosan. A comparison of the results obtained using these formulations compared to an ointment preparation containing the same active ingredients is shown in Fig. 4.

DISCUSSION

Both ethylcellulose and polyamide films were found to be suitable for use with either neomycin or triclosan. The films were flexible and clear. In addition, when cast upon a substrate of mercury, a continuous film was formed.

Different results were obtained when one incision was made on each animal instead of two incisions. It is probable that there is some absorption of the active ingredient or test agent into the bloodstream. The agent may be transported *via* the circulatory system to the other incision and alter the results. For example, when the spray product containing neomycin sulfate was applied to the left incision on the back of the guinea pig and 0.01 ml of the bacterial suspension was applied to the right incision, only seven out of 10 incisions on the right side became infected while four of the 10 animals developed an infection on the left incision. These responses were different when the study was repeated with only one incision and one agent per animal. In these cases, all 10 animals inoculated with the bacterial suspension became infected and only three of the 10 animals developed an infection when the neomycin spray was used on the incision.

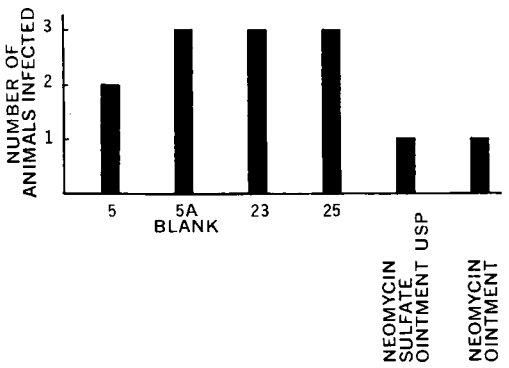

Figure 4—*Effectiveness of aerosol formulations* versus *ointments. Key: 5, neomycin sulfate with ethylcellulose; 5A, neomycin sulfate with polyamide resin; 23, triclosan with ethylcellulose; and 25, triclosan with polyamide resin.*

These results seem to indicate that the bacterial suspension applied to the right incision entered the bloodstream and contaminated the left and/or the aerosol spray applied to the left incision entered the bloodstream to reduce the total number of infected incisions on the animal's right side. Based on this possibility, the *in vivo* tests in this study were conducted using only one incision per animal in an effort to obtain more reliable results. In addition, only one agent was tested per animal. In previously cited studies (12–14), two incisions were made on each animal and then each incision was dealt with in a different way. The results obtained from those studies should be viewed with the possibility of cross-contamination between the two incisions.

As can be seen from Figs. 2–4, neomycin sulfate and triclosan were released from the ethylcellulose and polyamide films. While no spray was 100% effective in eliminating an infection, similar results were obtained with the controls and other substances studied such as ethanol, isopropyl alcohol, and an ointment prepared from neomycin or neomycin sulfate. However, when an infection did develop in the presence of the antibiotic or anti-infective, the degree of the infection was usually less intense, indicating that the drugs were released from the films and that the products did possess some degree of effectiveness. A higher concentration of therapeutic agent might be necessary to control the infection completely. While it was difficult to discern several borderline results, for the most part the method used was effective in differentiating the effects of different formulations.

An attempt was made to correlate the skin temperature on the 1st and 4th postoperative days with the degree of infection, but valid results were not obtained. Several factors interfered with obtaining an accurate skin temperature including ambient room temperature, the state of excitement of the animal, and the basal metabolic state of the animal. The thermographic tape used was obviously of too great a range for the detection of the fairly small temperature changes noted. Further studies are underway to modify this procedure by using a different range of thermographic tape.

Finally, no difference was noted in the results obtained using either the polyamide or ethylcellulose as the film former. The same held true for the plasticizers.

SUMMARY AND CONCLUSIONS

The formulation of a topical antibiotic spray-on bandage was attempted to develop an effective preparation for treating contaminated wounds. Many varied formulations were prepared using neomycin sulfate and triclosan as the anti-infective agents; ethylcellulose and polyamide resins served as the film-forming agents, and tributyl citrate and hexadecyl alcohol were chosen as the plasticizers.

Standardized, contaminated wounds in guinea pigs served as the easily reproducible laboratory *in vivo* testing system. The inflammatory response of the wounds was evaluated using several indexes including skin temperature, area of induration about the incision, presence or absence of pus, and color of the thermographic liquid crystal tape applied to the wounded area.

This method was effective in evaluating the *in vivo* release of therapeutic agents from a film. The results indicate that neomycin sulfate and triclosan were released from their formulations. The spray-on bandages did reduce the degree of infection about the wound. No difference was noted between the results obtained

[10] Hoffmann–La Roche, Inc., Nutley, N.J.
[11] Tele-Thermometer.

using either film former. The skin temperature of the animal and the use of a liquid crystal thermographic tape did not prove to be useful evaluative tools because additional factors may interfere with obtaining a true reading.

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Conformationally Constrained Analogs of Mescaline

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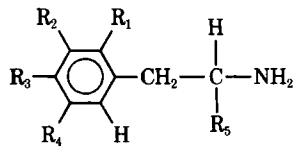
Abstract □ The syntheses of 3-(3,4,5-trimethoxyphenyl)piperidine, 2-(3,4,5-trimethoxybenzyl)piperidine, and 2-(3,4,5-trimethoxyphenyl)morpholine are described. In addition, preliminary pharmacological data comparing these compounds with mescaline are given.

Keyphrases □ Mescaline—synthesis of conformationally constrained analogs, pharmacological data □ Hallucinogens—synthesis of conformationally constrained analogs of mescaline, pharmacological data

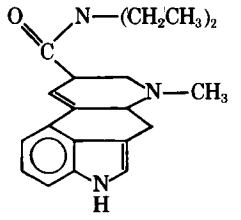
Recent years have seen extensive research in the synthesis of hallucinogens. Most of these compounds have been related to mescaline (I) and methoxyamphetamines (II) rather than to the more complex lysergic acid diethylamide (lysergide, LSD) (III) and tetrahydrocannabinol (IV) analogs (1). The latter two compounds are fairly rigid molecules; however, mescaline's side chain is conformationally mobile.

Most modifications of mescaline have been on the aromatic ring (1–3). However, Walters and Cooper (4) prepared *trans*-2-(3,4,5-trimethoxyphenyl)cyclopropylamine (V) in which the side chain was placed in a cyclopropyl ring. This analog produced mescaline-like activity. Later, Cooper (5) synthesized VI, the *cis*-isomer of V, and Trager and Huitric (6) prepared two related compounds, *cis*- and *trans*-2-(3,4,5-trimethoxyphenyl)cyclohexylamine (VII and VIII) by placing the side chain in a cyclohexyl ring.

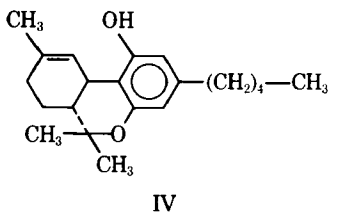


I: $R_1 = R_5 = H$, $R_2 = R_3 = R_4 = OCH_3$

II: R_1, R_2, R_3, R_4 = H or OCH_3, $R_5 = CH_3$



III



IV

The purpose of this investigation was to impose conformational restraint on the aliphatic side chain of mescaline by placing it in various heterocyclic ring systems, namely piperidine and morpholine. This report discusses the synthesis of 3-(3,4,5-trimethoxyphenyl)piperidine (IX), 2-(3,4,5-trimethoxybenzyl)-piperidine (X), and 2-(3,4,5-trimethoxyphenyl)morpholine (XI).

DISCUSSION

The procedure of Telang and Smith (7) was employed to synthesize 3,4,5-trimethoxyphenylacetonitrile (XII). Compound XII was