Effect of a Synthetic Dressing Formed on a Burn Wound in Rats: a Comparison of Allografts, Collagen Sheets, and Polyhydroxyethylmethacrylate in the Control of Wound Infection

PAUL NATHAN, BRUCE G. MACMILLAN, AND IAN A. HOLDER

Shriner's Burns Institute and Departments of Physiology, Surgery, and Microbiology, University of Cincinnati College of Medicine, Cincinnati, Ohio 45219

Received for publication 15 May 1974

Allograft dressings to control Pseudomonas wound infections in rats were studied on surgical wounds and escharectomized burn wounds. The effects of allografts were compared with a collagen sheet (Aviderm) and a synthetic dressing, polyhydroxyethylmethacrylate (Hydron), formed on the wound by mixing the polymer and the solvent. The results indicated that infections in surgical wounds were more easily controlled by dressings than similar contaminations in burn wounds. A procedure was described for the formation of a synthetic dressing directly on the wound from a mixture of polymer and solvent. This type of preparation completely filled the wound area and sealed the edges, preventing further contamination, and gave excellent coverage of the wound. With 24 h of coverage of escharectomized burn wounds, allografts provided the best dressing for reduction of wound organisms. At 96 h of coverage, Hydron and Aviderm produce significant reductions in the Pseudomonas resident in the burn wound. The results support the thesis that suitable dressings promote local host defense processes which kill the contaminating bacteria.

Previous work (1–4, 6) has demonstrated that some synthetic dressings applied to contaminated, full-thickness, surgical wounds approach the effectiveness of xeno- or allografts in their ability to promote control of the surface contamination. The present report extends these observations to experimental burn wounds on which the synthetic dressing is formed by addition of polymer and solvent to the surface of the escharrectomized burn wound. We also report the effects of sheets of bovine collagen (Aviderm) and skin allografts on the control of burn wound infection.

The results show that a Pseudomonas aeruginosa-contaminated burn wound presents a more difficult situation for treatment than a similarly infected surgical wound. However, under some conditions, both synthetic and natural-product dressings proved effective in aiding the host defense against bacterial contamination of the burn wound.

MATERIALS AND METHODS

Animals. Adult rats (Sprague-Dawley) weighing 200 to 300 g were anesthetized by intraperitoneal injections of pentobarbital, and their backs were shaved. The rats were anesthetized for all subsequent procedures.

Experimental surgical wound. A piece of skin (2.5 cm²) was removed from the rats' back, leaving the underlying panniculus carnosus muscle undisturbed. The exposed panniculus muscle was inoculated with 10⁷ P. aeruginosa taken from an actively growing 18-h culture. Details of the procedure have been published previously (5). To permit establishment of wound infection, the graft materials tested were applied to the pannicular surface 24 h after wound contamination with P. aeruginosa. Graft and test dressings were covered with Telfa pads and protected as previously described (5).

Burn wounds. The anesthetized rats were held in a metal device (Fig. 1) so that about 10% (25 cm²) of the body surface was exposed to hot water (95 C) for 10 s. A standard device for holding the rats was modified by application of 1/4 inch (ca. 0.64 cm) of vulcanized rubber to the entire unit to prevent second-degree burns from the hot metal. Also, a section of polyethylene tubing was glued along the length of the opening. This helped seal out the hot water which would otherwise run along the rats' skin, producing a second-degree burn of indeterminate size.

Graft materials. Full-thickness rat allograft skin was applied to infections established for 24 h on the exposed panniculus muscle. Aviderm (Avicon Inc., Fort Worth, Texas), a sheet of sterile bovine collagen, was applied as a dressing. In another set of animals, the synthetic Hydron (polyhydroxyethylmethacrylate, supplied by Hydro Med Sciences, Inc., New York) was formed as a sheet on the infected pan-
niculus. First 0.5 ml of polyethylene glycol was spread on the wound (Fig. 2A). Then the polymer in a powder form (0.5 g) was added to saturate the liquid (Fig. 2B). A solid film, the synthetic, formed on the wound and overlapped the edges of normal skin so that a firm seal was established over the exposed tissue (Fig. 2C).

Bacterial culture and septic wound model. A culture of *P. aeruginosa* was prepared as previously described (5). The dressing materials evaluated were applied at 1 or 7 days after contamination. Several protocols were used to vary the time from burn to either contamination or escharectomy and dressing application. These schedules are detailed below.

The test dressings were: (i) autografts of rat skin, (ii) collagen sheets (Aviderm) split from cow skin and modified so that it was largely collagen, and (iii) polyhydroxyethylmethacrylate film prepared by mixing polyethylene glycol and a powder of the polymer so that a film formed on the wound.

Quantitation of tissue bacterial content. At varying times (listed below) the dressings were removed, their underlying contaminated muscles were homogenized, and their bacterial content was determined as previously described (5). The bacteria contained in the muscle were expressed as the mean log_{10} (log_{10}) of *P. aeruginosa* per gram of muscle.

Statistics. Significance of individual treatments was determined by the Student's *t* test. Data were considered significant when the means differed at a probability less than or equal to 0.05.

RESULTS

A full-thickness surgical wound was prepared, and the exposed muscle was contaminated. Groups of the rats were treated in three ways: (i) no dressing was applied to one set of animals; (ii) autografts were used to cover another set of wounds; and (iii) the Hydron film was formed on the wounds of the third group. The first model tested was the immediately contaminated surgical wound (Table 1). When no dressing was applied to the wound the log_{10} of *P. aeruginosa* per g of muscle was 7.79 two days after contamination. Autografts produced a reduction in the log_{10} of *P. aeruginosa* per g of muscle to 6.77 (probability was not significant). A Hydron film dressing produced a significant reduction in the log_{10} of bacteria per g of muscle to 5.69 (*P < .001*).

In the next set of experiments an established

**Fig. 1.** Device for holding and exposing part of the anesthetized rats' back to hot water. All of the metal is covered with a thick layer of vulcanized rubber. The edge of the opening has a layer of plastic to act as a seal to prevent leakage of hot water onto other areas of the rats' skin.
infection was produced by contaminating the surgical wound and allowing 1 day for the bacteria to invade the wound (Table 2). The dressing, autografts, or Hydron film were applied, and after an additional day bacteria in the muscle were quantitated. The controls, without dressing, had a log_{10} of bacteria per g of muscle of 7.89. The autografts and Hydron significantly reduced the bacteria in the muscle from the control values to log_{10} 6.31 and 6.42, respectively.

Previous unpublished experiments in our laboratory by Artur A. Burget indicated that bovine collagen (Aviderm) was also effective in promoting host control of bacterial infection of surgical wounds. In view of these results and observations reported in Tables 1 and 2, we decided to extend the investigation to study the effect of Hydron and Aviderm on contaminated burn wounds. In the first set of tests the burn wound surface was contaminated, and 1 day later the eschars were removed and the test dressings were applied to the exposed muscles.

The log_{10} of P. aeruginosa per g of muscle (Table 3) in the group with no dressing was 7.76. Covering the contaminated muscle with autografts reduced the log_{10} bacterial level to 5.60. In contrast, neither Hydron film dressings nor Aviderm significantly lowered the wound bacteria when the results were compared to the controls without dressings.

**Table 1. Effect of dressing on immediately contaminated surgical wound**

| Treatment         | Mean | n  | SEM | P    |
|-------------------|------|----|-----|------|
| No dressing       | 7.79 | 6  | 0.27|      |
| Autograft         | 6.77 | 7  | 0.41| NS   |
| Hydron film       | 5.69 | 5  | 0.34| <.001|

* Wounds were contaminated and dressings applied on day 0, and muscle quantitation was performed on day 2. Results are expressed as log_{10} of P. aeruginosa per gram of muscle at 48 h. SEM, Standard error of the mean; NS, not significant.

**Table 2. Effect of dressing (24 h) on established infection of surgical wound**

| Treatment         | Mean | n  | SEM | P    |
|-------------------|------|----|-----|------|
| No dressing       | 7.89 | 6  | 0.56|      |
| Autograft         | 6.31 | 6  | 0.22| <.05 |
| Hydron film       | 6.42 | 9  | 0.21| <.005|

* Wounds were contaminated on day 0, dressings were applied on day 1, and muscle quantitation was performed on day 2. Results are expressed as log_{10} of P. aeruginosa per gram of muscle at 48 h. SEM, Standard error of the mean.

Since, clinically, dressings are usually left in place for several days, in another set of tests we waited 4 days before removing the dressings from the burn wound. At this time, the log_{10} of bacteria per g of muscle was 9.60 for the control series of rats with no dressing applied (Table 4). The rats with skin autografts had a log_{10} of 7.96. Hydron film gave a log_{10} of 8.46, and Aviderm dressings gave a log_{10} of 8.99. All the dressings had significant effects on the wound contamination; however, skin grafts were most effective.

To obtain a situation more closely reproducing the events in a clinical situation, the model described in Table 5 was developed. Seven days after a scald burn, the eschar was contaminated with P. aeruginosa. On day 14 after the burn and 7 days after contamination, the eschar was removed and the test dressings were applied. On day 18 the dressings or eschar were removed and the bacteria in the muscle were quantitated. An additional group of rats was studied (eschar in place) in which the eschar was contaminated on day 14 and left undisturbed until day 18. The control group with no dressing applied (Table 5) had a log_{10} of P. aeruginosa per g of muscle of 8.86. When the eschar was left in place the log_{10} of bacteria per g of muscle was.

**Table 3. Effect of 24-h dressing on contaminated burn wound**

| Treatment         | Mean | n  | SEM | P    |
|-------------------|------|----|-----|------|
| No dressing       | 7.76 | 8  | 0.33|      |
| Allograft         | 5.60 | 9  | 0.45| <.005|
| Hydron film       | 7.19 | 11 | 0.34| NS   |
| Aviderm           | 8.10 | 9  | 0.39| NS   |

* Burning and contamination were performed on day 0; eschars were removed and dressings were applied on day 1. Bacterial quantitation was performed on day 2. Results are expressed as log_{10} of P. aeruginosa per gram of muscle. SEM, Standard error of the mean.

**Table 4. Effect of 96-h dressings on contaminated burn wounds**

| Treatment         | Mean | n  | SEM | P    |
|-------------------|------|----|-----|------|
| No dressing       | 9.60 | 8  | 0.22|      |
| Allograft         | 7.96 | 5  | 0.32| <.005|
| Hydron film       | 8.46 | 9  | 0.38| <.05 |
| Aviderm           | 8.99 | 15 | 0.13| <.025|

* Burning and contamination were performed on day 0; eschars were removed and dressings were applied on day 1. Bacterial quantitation was performed on day 5. Results are expressed as log_{10} of P. aeruginosa per gram of muscle. SEM, Standard error of the mean.
Table 5. Effect of dressing on burn wound after extended period with eschar in place

| Treatment | Determination | Mean | n | SEM | P |
|-----------|--------------|------|---|-----|---|
| No dressing       | 8.96 | 4 | 0.41 |
| Eschar in place*  | 7.62 | 5 | 0.53 | .02 |
| Allograft         | 5.65 | 4 | 0.58 | <.005 |
| Hydron film       | 5.29 | 5 | 0.59 | <.005 |
| Aviderm           | 6.53 | 4 | 0.19 | <.005 |

* Burning was done on day 0; contamination was performed on day 7; eschars were removed and dressings were applied on day 14; and bacterial quantitation was performed on day 18. Results are expressed as log_{10} of P. aeruginosa per gram of muscle. SEM, Standard error of the mean.

In this experiment, the eschar was contaminated on day 14 and left undisturbed until day 18.

7.62 (probability was not significant). Allografts, Aviderm, and Hydron dressings all resulted in significantly reduced bacterial levels in the wounds. Wounds covered by dressings had 1/100 to 1/1000 the bacteria present in the control animals.

DISCUSSION

The results presented demonstrate a distinct difference between the burn wound and a full-thickness surgical wound of the skin. For example, the synthetic, Hydron, and the processed bovine collagen, Aviderm, are effective dressings on immediate or established infections produced in surgical wounds. These dressings were not helpful in an established infection on a burn wound when the dressings remained in place only one day. Evidently the burn wound presents a more demanding situation for control of infection than a surgical wound, which is largely normal tissue.

This report shows that when the dressing application on a contaminated burn wound is extended to 96 h, Hydron and Aviderm both promote the host’s ability to control a local infection in the burn wound. In particular, the model in which the burn wound was contaminated on day 7 after injury and the dressing applied on day 14, Aviderm substantially reduced the bacterial count. Also, in this model the effect of Hydron was similar to that obtained with skin allografts.

It is worth noting that the formation of the solid dressing on the wound by mixing the solvent and the polymer is a new approach to wound dressings (Fig. 2). This synthetic dressing conforms to the wound and it seals the surface and edges to protect the wound from additional contamination as well as serving as a conventional dressing, helping to promote local defense against bacteria in the wound.

In general, the present observations agree with previous reports (1, 2, 4) that a variety of dressings can be helpful in covering the burn wound. In particular, a recent report by Robson and Krizek (4) showing that amniotic membrane is as effective as xenograft skin provides results similar to ours. These authors also note a sharp drop in wound bacteria after a 96-h dressing compared to 48 h of wound coverage.

Robson and Krizek (4) failed to demonstrate the presence of a biological material in amniotic membrane which killed bacteria in vitro. They concluded that wound coverage promoted local host defense mechanisms. Studies by Saymen et al. (6) have previously indicated that this local defense process may involve the leukocytes in the wound. Grafts as well as synthetics modified the migration pattern of the cells infiltrating the wound. This changing migration pattern was associated with destruction of a substantial portion of the invading bacteria in surgical wounds. Our results indicate that a synthetic dressing formed on the wound may also activate the local defense process to destroy P. aeruginosa bacteria contaminating the wound.

ACKNOWLEDGMENT

We thank Daniel Murphy for excellent technical help and for useful suggestions.

LITERATURE CITED

1. Chvapil, M., R. L. Kronenthal, and W. van Winkle, Jr. 1973. Medical and surgical applications of collagen, p. 1-43. In D. A. Hall and D. S. Jackson (ed.), International review of connective tissue research. Academic Press Inc., New York.

2. Guldalian, J., C. Jelenko III, D. Callaway, and J. T. McKnight. 1973. A comparative study of synthetic and biological materials for wound dressings. J. Trauma 13:32-35.

3. Nathan, P., I. A. Holder, and B. G. MacMillan. 1973. Burn wounds: microbiology, local host defenses and current therapy. Crit. Rev. Clin. Lab. Sci. 4:61-100.

4. Robson, M. C., and T. J. Krizek. 1973. The effect of human amniotic membranes on the bacterial population of infected rat burns. Ann. Surg. 177:144-149.

5. Saymen, D. G., P. Nathan, I. A. Holder, E. O. Hill, and B. G. MacMillan. 1972. Infected surface wound: an experimental model and a method for the quantification of bacteria in infected tissues. Appl. Microbiol. 23:509-514.

6. Saymen, D. G., P. Nathan, I. A. Holder, E. O. Hill, and B. G. MacMillan. 1973. Control of surface wound infection: skin versus synthetic grafts. Appl. Microbiol. 25:921-934.