Iodoform Gauze Removes Necrotic Tissue from Pressure Ulcer Wounds by Fibrinolytic Activity

Fumihiro Mizokami, Yusuke Murasawa, Katsunori Furuta, and Zenzo Isogai

Department of Pharmacy, National Center for Geriatrics and Gerontology; Department of Advanced Medicine, National Center for Geriatrics and Gerontology; Department of Clinical Research and Development, National Center for Geriatrics and Gerontology; and Division of Dermatology and Connective Tissue Medicine, National Center for Geriatrics and Gerontology; 35 Gengo, Morisoka-machi, Obu, Aichi 474–8511, Japan.

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Iodoform gauze is used in clinical practice for treatment of infected wounds. However, effectiveness and action mechanism of iodoform gauze for removal of necrotic tissue are unknown. We therefore employed case control and biochemical studies in order to clarify the pharmacological activity of iodoform gauze. A clinical study demonstrated that treatment with iodoform gauze removed necrotic tissue more effectively than treatment with conventional ointments. More than 60% of iodoform gauze-treated wounds were completely debrided within 2 weeks. Consistent with the clinical observation, biochemical analyses revealed clear differences in wound fluid proteins after treatment with iodoform gauze or conventional gauze. The amount of macroaggregates of type I collagen from wounds were remarkably decreased in iodoform gauze. Moreover, iodoform gauze and iodoform itself released non-aggregative type I collagen from necrotic debris in vitro. Taken together, we conclude that iodoform gauze efficiently removes necrotic tissue by its lytic activity for collagen fibers.

Key words iodoform gauze; pressure ulcer; collagen; necrotic tissue; debridement

Iodoform (triiodomethane, CHI₃) consists of yellow crystals or a crystalline powder with an irritating odor. Historically, Serulla discovered the chemical in 1822, and in 1843, Dumas announced its composition. In 1880, iodoform was first used in medical practice by Bouchardat. Currently, iodoform gauze (IG), composed of iodoform and gauze, is used for disinfection, based on the experience of clinicians. Iodoform, reduced by wound fluids, exhibits antimicrobial activity after topical application to wounds.

Debridement is essential for managing necrotic wounds such as arterial or venous leg ulcers, pressure sores, or burns. Several methods for wound debridement are available, for example, surgical excision of necrotic tissue, repeated application of moistened dressings (saline-soaked gauzes), hydrocolloid or semiocclusive dressings, dextranomers, intracavity gels, or various enzyme preparations. Surgical debridement is obviously the most effective method, however, it cannot always be performed in elderly patients because of the physical pain and mental stress. Therefore, chemical debridement by topical agents is required in practice. In the past 20 years, several enzymatic products for wound debridement, such as Elase (fibrinolysin/DNAse, Parke-Davis Pharmaceutical, Hoofddorp, the Netherlands) and Novuxol (collagenase, Knoll Pharmaceutical, Ludwigshafen, Germany), have been developed. However, those products are no longer available because of the lack of stability of the raw materials.

At the initial stage of a deep pressure ulcer, necrotic tissue is usually present within a wound. Necrotic tissue in a pressure ulcer wound usually consists of dermis, fatty tissue, fascia, tendon and ligament, which are abundant in collagenous extracellular matrix, consisting mainly of type I collagen. Since treatment of deep pressure ulcers is initiated by debridement, topical agents used for this initial stage are required to have lytic activity for collagenous tissues.

In the present study, we have demonstrated the effectiveness and the action mechanism of IG in a retrospective observation study and by biochemical analyses and conclude that IG debrides a wound through its collagenolytic activity.

MATERIALS AND METHODS

Wound Data Collection The study was conducted at the National Center for Geriatrics and Gerontology Hospital, (NCGG, Obu, Japan). NCGG provides general medical services including emergency, and admits approximately 5000 patients per year (more than 90% of patients are elderly, over 65 years old) in a 300 bed hospital facility. Patients with pressure ulcers are managed by a specialized team at least once a week.

A list of pressure ulcer patients with necrotic tissue was extracted from a pressure ulcer database of NCGG, which allowed the identification of potential study patients. In order to create a database, all patients with pressure ulcers were systematically recorded during a 2-year period (from June, 2008 through May, 2010). The percentage of the hospitalized patients with pressure ulcers ranged from 2.8 to 11.0% during the period. The size of every pressure ulcer was measured and photographed at least once a week. The depth of pressure ulcers was determined according to the criteria of NPUAP (National Pressure Ulcer Advisory Panel).

A retrospective observational study was conducted with wound-cleaning capacity as the primary outcome for 60 patients treated with IG or conventional ointment therapy during the past two years. The size of the wounds was measured at least once a week, and the area was calculated according to the Japanese guidelines. The clinical information about patients including laboratory data for thyroid function (5 patients) and their wounds was obtained from the medical records and digital photographs. The area of necrotic tissue was blindly determined using digitalized images, according to

The authors declare no conflict of interest.
the previously reported study. Thus, we determined the percentage of necrotic tissue and wound size by tracing the photograph using UTHSCSA image tool Ver3.0 (Department of Dental Diagnostic Science at The University of Texas Health Science Center, TX, U.S.A.) and Photoshop CS4 (Adobe Systems Inc., CA, U.S.A.).

During the observation period, iodoform gauze (Tamagawa Eizai, Tokyo, Japan) was applied with a polyurethane top dressing (Bioclusive: Johnson and Johnson Medical Inc., Arlington, TX, U.S.A.). The conventional treatments for this study were silver sulfadiazine cream and povidone-iodine sugar. Those topical agents are listed in the Japanese guidelines for pressure ulcer treatment and are widely used for pressure ulcers with necrotic tissue in Japan.

Statistical Analysis All comparisons were unpaired, and all tests of significance were two-tailed. Continuous variables were compared using the Student’s t-test for normally distributed variables and the Mann–Whitney’s U-test for non-normally distributed variables. The chi-square or Fisher’s exact tests were used to compare categorical variables. We performed analysis using a statistical software program (JMP, version 8.0 for Windows; SAS Institute Inc., Cary, NC, U.S.A.).

Biochemical Studies Using Wound Sulfate Proteins All protein samples in this study were routinely collected from pressure ulcer wounds at NCGG after written informed consent. IG and conventional gauze (CG) containing wound fluid were stored at −80°C until used. In some experiments, wound surface proteins were sampled by short contact with IG. This protocol was approved by the Ethics Committee of NCGG and complied with the ethical rules in the Declaration of Helsinki. Samples were chosen from stored gauze bandages that had been used for two consecutive days before IG treatment was started. Necrotic tissue was removed from wound surface by contact with CG after less-invasive surgical debridement. The gauzes were extracted with 6 M Gdn solution (6 M guanidine hydrochloride, 50 mM Tris–HCl, 1 mM phenylmethylsulfonyl fluoride, 1% (v/v) protease inhibitor cocktail (Sigma), pH 7.5) at 4°C for 72 h, and the supernatant was collected by centrifugation at 12000 rpm for 10 min. As a standard, pepsin-digested type I collagen was obtained from Nitta gelatin (Osaka, Japan).

Gel Filtration, Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) and Immunoblotting Protein samples dissolved in 6 M Gdn solution were separated on sepharose CL-2B gel chromatography under dissociative conditions as previously described. The eluted fractions were categorized in high molecular weight fractions (fractions 28–34; H), middle molecular weight fractions (fractions 62–68; L), as indicated in Fig. 1. The fractions were immobilized on nitrocellulose membranes by dot blotting. For Western blotting, protein samples were also precipitated by ethanol and resolved using 7.5% acrylamide SDS-PAGE under non-reducing conditions unless otherwise stated. The separated proteins on the gels were transferred onto nitrocellulose membranes (Bio-Rad, CA, U.S.A.). The membranes from dot blot or Western blot were blocked with 5% nonfat skim milk (Dako, Denmark) in Tris-buffered saline (TBS) containing 0.1% Tween-20 (TBST) at room temperature for 1 h, followed by incubation with anti-type I collagen antibody recognizing α1 chain (10 μg/mL, Abcam) in TBST containing 2% milk and with secondary antibody (Dako). The blots were developed by ECL (GE Healthcare, Uppsala, Sweden). The obtained signals by dot blot analyses were quantified by Image J software. Background was subtracted, and the integrated density of each dot was measured. The means±S.D. were calculated for each group in 3 independent analyses, and statistical analyses of the results were carried out by analysis of variance, followed by test of multiple comparisons to distinguish different groups.

In vitro Analyses Using Wound Attached Debris For in vitro analyses, necrotic tissues (~100 mg) were washed three times with phosphate buffered saline (PBS) and then incubated with IG (25 cm²) or control normal gauze (25 cm²) in 3 mL of PBS at 37°C for 24 h. After incubation, gauze and insoluble tissues were removed by centrifugation for 3 min at 3000× g. The supernatant was collected and the residual insoluble tissue and the gauze were extracted with 3 mL of 6 M Gdn solution at 4°C for 72 h. In some experiments, iodoform solution and PBS alone were also used for incubation with necrotic tissue. Iodoform solution was prepared by incubation of IG (25 cm²) with 3 mL of PBS at 4°C for 1 h.

RESULTS

Cases of Pressure Ulcer Wounds with Necrotic Tissue Treated with Iodoform Gauze Representative clinical findings of pressure ulcers treated with IG are shown (Fig. 1). In both cases, the necrotic tissue was removed by IG treatment within two weeks. The appearance of the granulation tissue was pinkish, suggesting abundant vessel formation.

Iodoform Gauze Efficiently Removes Necrotic Tissue Compared with Conventional Therapy In order to clarify the clinical effect of IG, a total of 60 wounds in 53 patients were analyzed. Thirty wounds were treated by conventional ointment and thirty other wounds received IG therapy. The detailed information is summarized in Table 1. The mean age

![Fig. 1. Clinical Appearance of Pressure Ulcer Wounds Treated with Iodoform Gauze](image-url)
Table 1. Characteristics of Patients Treatment with Iodoform Gauze or Conventional Ointment in This Study

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Iodoform gauze (n=27)</th>
<th>Conventional ointment (n=26)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>80.4±11.0 (44–98)</td>
<td>81.3±6.1 (74–96)</td>
<td>0.356</td>
</tr>
<tr>
<td>Gender (male/female)</td>
<td>16/11</td>
<td>9/14</td>
<td>0.128</td>
</tr>
<tr>
<td>Albumin (g/dL)</td>
<td>2.8±0.5 (1.4–3.8)</td>
<td>3.2±0.6 (2.0–4.2)</td>
<td>0.007**</td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>10.4±2.0 (5.2–14.2)</td>
<td>11.4±2.2 (5.2–14.5)</td>
<td>0.065</td>
</tr>
<tr>
<td>Nutrition</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oral</td>
<td>12</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>EN</td>
<td>3</td>
<td>2</td>
<td>0.126</td>
</tr>
<tr>
<td>PPN</td>
<td>11</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>TPN</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Charlson Index</td>
<td>3.2±1.5 (1–7)</td>
<td>3.2±1.4 (2–8)</td>
<td>0.489</td>
</tr>
<tr>
<td>Barthel Index</td>
<td>12.4±21.2 (0–85)</td>
<td>14.7±30.0 (0–100)</td>
<td>0.387</td>
</tr>
</tbody>
</table>

** (p<0.01) Indicates a statistically significant difference as determined by the Student’s t-test.
Data are presented as the mean±standard deviation (minimum–maximum). EN: enteral nutrition, PPN: peripheral parenteral nutrition, TPN: total parenteral nutrition.

Table 2. Wound Characteristics in This Study

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Iodoform gauze (n=30)</th>
<th>Conventional ointment (n=30)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surface area (cm²)</td>
<td>17.6±19.6 (1.4–65.0)</td>
<td>7.7±8.2 (0.3–32.0)</td>
<td>0.004**</td>
</tr>
<tr>
<td>Necrotic tissue as a percentage of total surface area (%)</td>
<td>85.8±14.4% (14–100)</td>
<td>84.4±21.2 (20–100)</td>
<td>0.361</td>
</tr>
<tr>
<td>Depth (NPUAP stage)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grade II</td>
<td>0 (0%)</td>
<td>3 (10%)</td>
<td></td>
</tr>
<tr>
<td>Grade III</td>
<td>5 (16.7%)</td>
<td>10 (33%)</td>
<td>0.009**</td>
</tr>
<tr>
<td>Grade IV</td>
<td>25 (83.3%)</td>
<td>17 (57%)</td>
<td></td>
</tr>
<tr>
<td>Parts</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sacrum</td>
<td>16</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Trochanter</td>
<td>3</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Heel</td>
<td>4</td>
<td>4</td>
<td>0.070*</td>
</tr>
<tr>
<td>Other</td>
<td>7</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>Treatment period (days)</td>
<td>14.1±9.7 (6–40)</td>
<td>29.0±24.5 (7–100)</td>
<td>0.002**</td>
</tr>
</tbody>
</table>

* (p<0.05), ** (p<0.01) indicates a statistically significant difference as determined by the Student’s t-test.
Data are presented as the mean±standard deviation (minimum–maximum).
Fig. 2. Reduction of Necrotic Tissue in Pressure Ulcer Wound Treated with Iodoform Gauze or with Conventional Ointments

Necrotic tissue was evaluated by analysis of the photographs. The reduction rate of necrotic tissue was calculated compared with day 0. Filled squares indicate a group treated with iodoform gauze (n=30) and blank triangles indicate a group treated with conventional ointments (n=30). Necrotic tissues treated with iodoform gauze decreased markedly. A statistically significant difference was observed between the two groups. *p<0.05.

Fig. 3. Reduction of Necrotic Tissue by Treatment with Iodoform Gauze

Percentage of wounds from which necrotic tissue was completely removed by treatment with iodoform gauze (n=30) or conventional ointment (n=30) and using compared with Fisher's exact tests. The success rate of treatment with iodoform gauze was over 60% after two weeks.

Fig. 4. Characterization of Type I Collagen Macromolecules from Pressure Ulcer Wounds with Necrotic Tissue

A, Extracts from iodoform gauze (IG) and conventional gauze (CG) were obtained from identical wound and were sieved on sepharose CL-2B chromatography under dissociative conditions. The protein concentrations of the fractions and dot blot analysis by anti-type I collagen antibody are shown. Vo indicates void volume. The fractions were categorized as H, M and L according to the eluted fractions, as described in Materials and Methods. B, Intensities of the dot blotting of the categorized fractions were quantified by image J. A significant difference between signals in fraction H is shown. *p<0.05. C, IG with wound fluid was analyzed by Western blotting analyses with anti-type I collagen antibody. Analyzed samples were chosen on the indicated days after initiation of IG gauze treatment. Positive bands corresponding to collagen β, γ and α1 isoforms of type I collagen are indicated by arrowheads. Control porcine collagen I obtained by pepsin digestion are shown as Col Ip. D, Wound surface proteins were analyzed by Western blotting with anti-type I collagen antibody. Samples used in this experiment were collected on two consecutive days before IG treatment (+) or right after IG treatment (+).
Signal intensity for type I collagen at the fractions H from multiple samples was significantly reduced in the IG extract (Fig. 4B). The molecular composition of the collagen from wounds was analyzed by Western blotting in comparison with pepsin-digested standard collagen type I. All species of type I collagen that corresponded to monomer (α1), dimer (β1) and trimer (γ1) were observed (Figure 4C, arrowhead). Those species of type I collagen were constantly detected on any day during treatment (Fig. 4C). By Western blot analysis of wound surface proteins, non-aggregative type I collagen was predominantly observed in IG treated wounds compared with CG treated wounds (Fig. 4D). In addition, degradative product of type I collagen was observed around 50 kDa consisting with previous study.13)

**Iodoform Gauze Releases Smaller Molecules of Type I Collagen from Wound Debris**

We next tested whether smaller molecules of type I collagen were released from necrotic tissues by the direct effect of IG in vitro. Necrotic debris was incubated with IG or CG in vitro, and was then extracted with 6 M guanidine hydrochloride. By gel filtration under dissociative conditions, the tissue extract incubated with CG contained macro-aggregates of type I collagen, whereas the extract pre-incubated with IG contained only middle and lower molecular weight species of collagen I (Fig. 5A). This result indicated the lytic activity for collagen fiber by IG, and was consistent with the data obtained from gauze used on wound surface. Further analyses by Western blotting demonstrated that the released type I collagen was attached onto the IG, and was not present in the soluble fraction (Figs. 5B, C). When necrotic debris was incubated with iodoform solution alone, (see Materials and Methods), non-aggregative type I collagen molecules were released into the solution (Fig. 5D).

**DISCUSSION**

In the present study, it is demonstrated that IG treatment is effective for wound bed preparation of deep pressure ulcers at the initial stage. This case control study showed that debridement by IG was much faster than the conventional ointment therapy currently prescribed in Japan. Moreover, most of the examined wounds were completely cleaned by IG without abnormal findings. This clinical observation suggests that IG can be used for clearing the debris from wounds. Although a randomized control trial with IG would be desirable, there would be difficulties connected with conducting a blind study because of a lack of appropriate comparison subjects in Japan and risk of further infection. Therefore, we performed biochemical studies to support the clinical findings.

Maggot therapy has been utilized for debridement of chronic ulcers, however, the cost and discomfort of the therapy...
are major reasons for not using it. In addition, public health insurance in Japan does not cover the treatment. Although another enzymatic debridement using collagenase appears to be effective, it is unavailable in Japan.10 Topical ointment with an emulsion base promotes autolysis of necrotic tissue, but the procedure is usually slow. In addition, autolysis sometimes complicates soft tissue infections. For those reasons, an effective topical therapy that actively removes necrotic tissue is required in the medical care practice. Therefore, IG, which is available in Japan and is also inexpensive, can be recommended in terms of both wound bed preparation and economic aspects.

In this study, we have not observed any adverse effects based on the physical findings and routine blood analyses. There are some case reports in which plasma iodine levels were elevated after iodiform intoxication.15 In addition, the iodine itself is known to cause nausea and liver and kidney dysfunction, as well as other disorders.16,17 Therefore, the maximum dose was reported to be 2 g.18 In our usage, the maximum dose of iodiform was calculated to be 0.33 g.

Consistent with the clinical studies, biochemical analyses of wound surface proteins showed that application of IG remarkably reduced the size of the macromolecules containing collagen I in wound surface proteins. Since type I collagen is assembled into collagen fibers in connective tissue, necrotic tissue of collagen-rich tissue, including fascia and tendons, contains type I collagen.9 Histopathological studies also reported the degeneration of collagenous tissue in pressure ulcer wounds.19 Our methods, using gel filtration under dissociative conditions, enabled us to analyze collagen-containing macromolecules that do not enter the SDS-PAGE gels. We first showed the debridement effect by the biochemical analyses of type I collagen from wounds.

The pharmacological effect of IG was also tested by in vitro analyses. Incubation of necrotic debris with IG released smaller sized collagen type I from necrotic tissues. It is also interesting that the majority of collagen molecules were attached to IG itself. Although the mechanisms of the release of type I collagen by IG is not clear at the present time, activation of some matrix degradation enzyme may be a candidate mechanism for the production of altered collagenous fibers shown in this study. Since this assay was performed on the stored necrotic tissue washed by PBS, an anti-bacterial effect of IG is not likely to be a major mechanism in the observed collagenolytic effect of IG. Collagen fibrinolysis in vitro may be a proper tool for testing the pharmaceutical effects of the topical agents.

Collectively, the effectiveness and action mechanism of IG provide new insight into the chemical debridement procedure at the initial stage of pressure ulcer wounds. Appropriate usage of IG accelerates the wound healing process of deep pressure ulcers with necrotic tissue through its collagen fibrinolytic activity.

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