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A Novel Property of Povidon-Iodine: Inhibition of Excessive Protease Levels in Chronic Non-Healing Wounds

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TO THE EDITOR

Chronic, non-healing skin wounds such as leg ulcers and pressure sores represent a significant clinical problem and a financial burden to health-care systems. For most patients, an underlying disease interferes with normal tissue repair, resulting in chronic wounds, which do not heal as expected. At the molecular level, excessive levels of matrix metalloprotease (Wysocki *et al.*, 1993), elastase activity (James *et al.*, 2003), and plasmin activity (Palolahti *et al.*, 1993; Lauer *et al.*, 2000) in wound fluid are characteristic of non-healing wounds. The excessive protease activities degrade growth factors (Trengove *et al.*, 1999; Lauer *et al.*, 2000) and newly formed extracellular matrix (Wysocki and Grinnell, 1990) and thus contribute to perturbation of tissue repair. Eventually, the wound becomes deadlocked.

Clinical evidence is controversial (Kjolseth *et al.*, 1994; Vogt *et al.*, 2001) but some reports suggest that aqueous polyvinyl pyrrolidone (PVP, povidon)-iodine preparations can promote tissue repair in non-healing wounds although the mechanisms remain unclear (Vogt *et al.*, 2001). Hypothesizing that PVP-iodine preparations reduce protease activity, we tested the effect of different PVP-iodine doses on the activity of selected proteases, which have been proposed to be critically involved in the pathophysiology of chronic non-healing skin ulcers (Palolahti *et al.*, 1993; Wysocki *et al.*, 1993; James *et al.*, 2003). Analysis was performed in wound fluids, which were obtained from different patients ($n=7$) presenting with chronic non-healing venous leg ulcers. For fluid collection, wounds were covered with a semipermeable polyurethane film for maximum 4 hours (Hydrofilm®, Hartmann; Heidenheim, Germany); fluids were collected, centrifuged (10 minutes, $13{,}000 \times g$, 4°C), and frozen (−80°C) until use. Protease analysis verified highly elevated levels of neutrophil elastase and plasmin activity in wound fluids (Table 1). Neutrophil elastase activity in serum of patients suffering from chronic wounds was below 1.5 mU/ml, confirming the local synthesis of elastase activity within the chronic wound environment.

Table 1. Protease activity in wound fluid obtained from venous stasis ulcers

Wound fluid	Elastase activity[1] (mU/ml)	Plasmin activity[1] (mU/ml)
No. 1	41	202
No. 2	34	63
No. 3	131	45
No. 4	312	68

[1]Protease activity is normalized to total protein concentration.

First, we tested the effect of different PVP-iodine doses on metalloprotease activity. After development, the metalloproteases appear as transparent bands in the gelatin zymography. There was a clear reduction of metalloprotease activity in PVP-iodine-treated wound fluids (Figure 1). At 27×10^{-5} M of diluted PVP-iodine stock/10 μg total protein, metalloproteinase activity was strongly inhibited and inhibition was still noticeable at 14.7×10^{-5} M of diluted PVP-iodine stock/10 μg total protein. We next analyzed inhibition of neutrophil elastase and plasmin activity with PVP-iodine. Already low doses of PVP-iodine could significantly inhibit purified plasmin and neutrophil elastase activity (Figure 2a, $^*P<0.001$). When tested in wound fluid, there was a clear dose-dependent inhibition of neutrophil elastase activity by PVP-iodine (Figure 2b). For plasmin, the inhibition was less pronounced, and in some samples almost missing. These data indicate some sort of substrate specificity for PVP-iodine action.

How can we explain our surprising observations? PVP-iodine is a powerful disinfectant with a broad microbicidal spectrum, long history of use, and absence of resistance development (reviewed by Fleischer and Reimer, 1997). Microbicidal activity of PVP-iodine at low concentrations becomes attenuated in the presence of high protein concentrations. Iodine is strongly oxidative and easily reacts with the amino, phenol, and –SH groups of amino acids,

Abbreviation: PVP, polyvinyl pyrrolidone

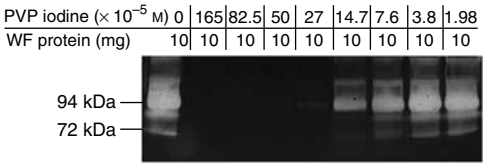

Figure 1. PVP-iodine inhibits metalloprotease activity in wound fluid obtained from non-healing wounds. A 10 μg portion of total wound fluid protein (Pierce BCA assay, through Perbio Science, Bonn, Germany) was incubated with 4 μl of PVP-iodine serial dilutions as indicated (Mundipharma GmbH, Limburg, Germany, or B Braun, Melsungen, Germany) in a total volume of 20 μl. These dilutions of PVP-iodine stock reflect the clinical practice of diluting PVP-iodine stock before application. After 30 minutes at room temperature, metalloprotease activity was revealed by gelatin zymography. Treated wound fluid was electrophoresed in gelatin-containing SDS gels. After washing, the renatured metalloproteases degraded gelatin and appeared as clear, transparent bands after Commassie staining. Wound fluid without PVP-iodine treatment is shown on the left lane. At PVP-iodine concentrations of 27×10^{-5} M/10 μg wound fluid protein, metalloprotease inhibition was very strong and even at 14.7×10^{-5} M/10 μg still noticeable; the zymogram is representative of additional wound fluids tested; WF, wound fluid.

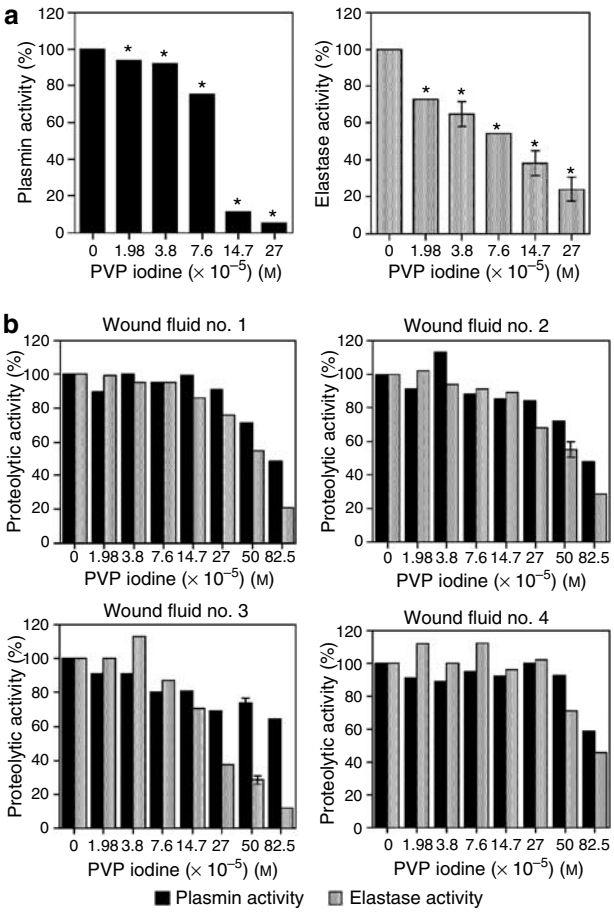

Figure 2. PVP-iodine inhibits elastase and plasmin activity in wound fluid obtained from non-healing wounds. (**a**) Human plasma plasmin (0.2 U/ml) (Roche, Mannheim, Germany) or human neutrophil elastase (60 mU/ml) (Merck Biosciences, Darmstadt, Germany) or (**b**) wound fluids obtained from four different patients (wound fluid nos. 1–4) was incubated with indicated concentrations of PVP-iodine at room temperature for 30 minutes. Plasmin activity or neutrophil elastase activity was detected by a plasmin (H-D-Val-Leu-Lys-AMC, Bachem, Weil a Rhein, Germany) or a neutrophil elastase (MeOSuc-Ala-Ala-Pro-Val-AMC, Bachem) specific fluorescent peptide substrate; for analysis, equal volumes of protease or wound fluid, PVP-iodine, and substrate solutions were added; protease activity was detected by measuring the fluorescence at $\lambda_{ex} = 360$ nm and $\lambda_{em} = 465$ nm in a spectrofluorometer (Tecan Fluorescensreader Spectrafluor). Analysis was performed twice and samples were assayed in triplicate; results are shown ±SD; an unpaired Student's *t*-test was used to analyze differences between groups; $^*P < 0.001$. Analyses are representative of additional wound fluids tested.

with unsaturated fatty acids and nucleotides. For enzymes, this results in denaturation and loss of function and we suspect that it is this mechanism we observe in our experiments. In principle, this is a general mechanism not specific for particular enzymes; however, the differences in plasmin and elastase inactivation suggest that some active centers are more susceptible to iodine radical inactivation than others. Nevertheless, our results suggest a broad spectrum activity suitable for the diverse and complex environment of non-healing wounds.

PVP-iodine treatment of non-healing wounds aims to reduce bacterial colonization (Mandy 1985). Although the effects on wound sanitation are limited from clinical experience as most bacteria persist, PVP-iodine use became controversial as *in vitro* research showed marked cellular toxicity and inhibition of normal wound healing in some animal trials (Lineaweaver *et al.*, 1985a, b; Cooper *et al.*, 1991; Smoot *et al*, 1991). Toxicity data for non-healing wounds appear more favorable (Reimer *et al.*, 2000; Vogt *et al.*, 2001, Fumal *et al.*, 2002). The high protein content in wound fluid may limit diffusion of iodine radicals into deeper parts of the wound and thus limit cell damage. We suggest using PVP-iodine in impaired wound healing when healing is poorly progressing, strongly exudating, and excessive protease levels predominate. Once this phase has given way to a healthy granulation tissue and epithelial migration occurs, this regimen may be switched to moist wound healing only.

From a clinical standpoint, this novel activity of PVP-iodine for non-healing wounds may explain the beneficial effects for impaired healing wounds. Our results may provide a treatment rationale suitable not only for those settings that can afford expensive modern wound dressings but also for less privileged areas where aqueous PVP-iodine may be readily available.

The study adhered to the Declaration of Helsinki Principles, was approved by the University of Cologne, and patient consent was received.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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