

Evaluation of hypochlorous acid washes in the treatment of chronic venous leg ulcers

- **Objective:** Hypochlorous acid (HOCl) is a highly microbiocidal agent active against bacteria, viruses and fungi. Using quantitative microbiology, preliminary studies showed it achieved an appreciable reduction in the bacterial burden in chronic venous leg ulcers. The study aimed to determine whether it has a role as an additional treatment for chronic venous ulcers that have not healed with conventional treatment.
- **Method:** On the basis of previous reports we designed a study in which patients acted as their own controls, in that only patients who failed to achieve a 44% reduction in wound size with standard treatment (compression bandaging) received HOCl washes.
- **Results:** Of 30 patients admitted to the study, 10 achieved a 44% ulcer reduction after three weeks of standard treatment. In addition to the standard compression treatment, the remaining 20 patients were given HOCl washes over 12 weeks. Of the 20 ulcers, nine (45%) healed and five (25%) reduced in size by over 60%. All patients became free of pain.
- **Conclusion:** These findings confirm the clinical efficacy of treating venous leg ulcers with hypochlorous washes. Use of HOCl washes as an adjunctive therapy for recalcitrant venous leg ulcers appreciably increases healing and rapidly relieves pain.
- **Declaration of interest:** This study was supported by a grant from Sterilox Technologies International, Stafford, England.

venous leg ulcers; hypochlorous acid; bacterial burden; ulcer healing

Hypochlorous acid (HOCl) is a highly active microbiocidal agent that is active against all bacterial, viral and fungal human pathogens.¹ Its rate of bactericidal activity is rapid, with a 'kill rate' for *Bacillus subtilis* spores of 10⁸ in one minute 28 seconds,² and it is considerably more bactericidal than hypochlorite solutions such as Edinburgh University Solution of Lime (Eusol).

HOCl has been shown not to produce skin sensitisation or irritation in animals, and not to be mutagenic in the Ames assay test.³ It has passed all the safety requirements for clinical use and, as Sterilox Tx (Sterilox Technologies International, Stafford, UK), is registered for use as a class 1/1B wound irrigation fluid under Directive 93/42/EEC and the medical device regulation 2002: 618.

Sterilox solutions have multiple-use specific clearances, including disinfection and wound irrigation in the UK and Europe.⁴ Machines suitable for producing Sterilox Tx will shortly be available.

Studies at the Churchill Hospital dermatology department's cell culture laboratory have shown that HOCl, specially formulated at pH5.4 at concentrations equivalent to those achieved when applied to humans, was not toxic to monolayer cultures of single-cell membranes, using fibroblasts and keratinocytes in foetal calf serum and Dulbecco's Modified

Eagles Medium (DMEM). Surprisingly, there was even stimulation of cell growth in some experiments.³

In view of these findings on the acceptability of this registered agent, in 2000 we undertook a pilot study involving six consenting patients with chronic lower leg venous ulcers. Ethics committee approval was not required as this product was registered for use as a wound irrigant.

We monitored occurrence of pain, discomfort or any other symptoms after exposure to HOCl. Bactericidal activity was measured by quantitative microbiological assessment of the ulcers' bacterial flora after immersion on two separate days, three days apart. This treatment yielded an appreciable reduction in the bacterial flora of the ulcers of 10²–10⁴ per treatment, exceeding 10⁶ after the two days. All six patients reported that the treatment did not cause pain during or after immersion in the solution. There was also a marked reduction in pain, especially at night, which had previously been most distressing to them.³

In view of these favourable results, we undertook a trial of this agent, used in combination with standard treatment, in the long-term treatment of chronic venous leg ulcers. Preliminary results were presented at the World Union of Wound Healing Societies, held in Paris in 2004 and published in a press release by Sterilox.⁴

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Design and method

Previous work has shown that complete healing was achieved by 12 weeks in 40% and 44% of patients with venous leg ulcers using short-stretch and four-layer bandaging respectively.⁵ A later prospective study reported that 65–70% of patients completely healed after 12 weeks of treatment with four-layer bandaging and 84–95% within one year.⁶ However, given the large number of patients with venous leg ulcers and the fact that, in 22% of patients, treatment failures or relapses will occur,⁷ improved treatments are clinically necessary.

It was not logistically possible to conduct a randomised controlled trial (RCT) of HOCl plus conventional compression bandaging (four-layer or short-stretch) due to the large number of subjects required. In addition, there is no suitable alternative antimicrobial agent to HOCl for comparison.

Attempts were thus made to restrict the controlled trial to patients with non-healing ulcers or who had experienced a recurrence. Unfortunately, over a six-month period this attempt failed as many patients refused to participate in a controlled trial because they were disillusioned with the standard alternative treatment (compression bandaging), which had previously failed them. Furthermore, possibly in view of their advanced age, they were disinclined to commit themselves to twice weekly clinic visits for the next six months for a treatment in which they had little confidence.

An unpublished study we had undertaken previously, following a suggestion made to us at a conference that water can be used as a comparator to HOCl, failed to demonstrate any measurable reduction in the quantitative microbiology of chronic venous ulcers following immersion into water for 20 minutes. This study was approved by the Central Oxfordshire Research Ethics Committee. We therefore considered it inappropriate to use water alone as a comparator in a controlled trial.

It was thus decided, on the basis of reports by Phillips et al.⁸ and Margolis et al.,⁹ to design the study so that the patients would be their own controls. Patients would be admitted for an initial three weeks of treatment with standard compression bandaging. According to Phillips et al.⁸ and Margolis et al.,⁹ healing can be expected to occur in only 22% of patients who failed to achieve a 44% reduction in wound size after three weeks' standard treatment. Our study protocol therefore stipulated that patients who did not achieve a 44% reduction in wound size at three weeks would be offered HOCl treatment (a HOCl wash for 20 minutes in a forced circulation leg hydrobath) in addition to the standard compression treatment.

Inclusion criteria were:

- Patients who had previously failed standard treatment with compression bandaging *with*

- An ulcer size of 5cm² or more *and*
 - An ulcer duration of at least six months
 - No insulin-dependent diabetes mellitus
 - Ankle-brachial pressure index (ABPI) ≥ 0.8 .
- There were no upper or lower age limits.

Thirty patients referred to the Department of Dermatology at Churchill Hospital took part. All gave consent. No difficulties in recruitment were experienced as most patients were willing to participate in a trial that offered an alternative to compression. Although sought, formal ethical approval was not required as HOCl is registered for wound irrigation.

Based on the work by Phillips et al.⁸ and Margolis et al.,⁹ we anticipated that at least 20 of the 30 patients would not achieve a 44% reduction in ulcer size with three weeks' conventional treatment and so would enter the second phase of the study.

The HOCl wash was administered twice a week for three weeks and then once a week for nine weeks, the reduced frequency being due to limited staff time. During the latter period patients continued to have routine dressing changes and reapplication of the compression bandage at home once a week.

After completing this programme of treatment patients were followed up for a further six to eight weeks while they continued with their standard compression bandaging treatment. This duration reflects the fact that healing can be slow in elderly patients.

The HOCl solution used was generated in the clinic using a proprietary electrochemical cell and control system, which converted by electrolysis a concentrated solution of salt to a solution consisting of over 97% HOCl at pH5.4–5.8, with a >950mV redox potential (Sterilox Technologies International, Stafford, UK). The concentration of HOCl used was 150–180 parts per million chlorine radicals.

At each visit, including all follow-up visits, the degree of pain was assessed using a modified McGill questionnaire,¹⁰ and the ulcers were photographed and traced. A sterile swab was pressed laterally on the wound to express underlying tissue fluid and exudate was taken for semiquantitative microbiology at the start of the treatment regimens and when deemed appropriate on clinical grounds. Routine blood samples were taken on admission for a biochemical screen and full blood count.

Based on the wound appearance, in particular erythema, increased exudate and odour, and supported by microbiological evidence of a heavy growth of pathogenic bacteria, topical antibacterial agents were applied during both phases of the trial when necessary. These were restricted to metronidazole, silver sulphadiazine, cadexomer iodine and Aquacel Ag (ConvaTec). The antibiotics were used sparingly, and when the data were analysed appeared not to have had a major therapeutic effect on their own.

Table 1. Ten patients who achieved a reduction in ulcer size of $\geq 44\%$ after three weeks' standard treatment with four-layer bandaging and appropriate local treatment

Initials/ age (yrs)	Size of ulcer on admission (cm ²)	Duration of ulcer (months)	Reduction of ulcer size after three weeks' standard treatment	Time to complete healing
MR/83	12.0	12	47%	Healed at 6 weeks
AD/49M	8.6	9	Nearly healed	Healed at 4 weeks
PH/84	13.5	6	Nearly healed	Healed at 5 weeks
CR/63	9.2	7	60%	Healed at 12 weeks
MH/84	6.4	5	48%	Healed at 11 weeks
CT/53	4.2	7	53%	Healed at 5 weeks
BG/88	8.8	12	60%	Increased in size due to MRSA infection and failure of dietary control of DM
MT/87	5.0	12	84%	Healed at 8 weeks
GR/63	3.1	7	48%	Healed at 8 weeks
HL/79	8.5	12	49%	Healed at 10 weeks

M = male; MRSA = methicillin-resistant *Staphylococcus aureus*; DM = diabetes mellitus

Systemic antibacterial chemotherapy was routinely restricted to the use of flucloxacillin orally for 10 days only for wounds that showed the presence of β -haemolytic streptococci of Lancefield groups A, B and G. This applied to only three patients with streptococcal group G infections.

The statistical analyses performed, including the Yates-corrected chi-square test, were restricted to the results for patients whose ulcers were $\geq 5\text{cm}^2$ and over six months' old.

Results

The results are set out in detail in Tables 1–4. Table 1 shows that all 10 patients who achieved a $\geq 44\%$ reduction in ulcer size after three weeks of standard treatment continued to improve, and nine (90%) achieved full healing within the 12 weeks of continued standard treatment. One patient who achieved a 60% reduction in ulcer size after three weeks of initial treatment further improved to an 80% reduction by nine weeks. However, this patient contracted methicillin-resistant *Staphylococcus aureus* (MRSA) in her wound which was associated with considerable enlargement of the ulcer and loss of dietary control of her diabetes mellitus. She was transferred to another department for insulin treatment and ulcer care, so was excluded from further follow-up.

The 20 remaining patients received HOCl as well as standard four-layer compression. The benefits of HOCl treatment are shown in Tables 2 and 3.

As shown in Table 2, in nine patients the reduction in ulcer size with three weeks of standard therapy

was less than 44% (mean reduction: 17%; range 0–34%). After HOCl treatment there was a substantial improvement (mean reduction after five weeks: 58%; range 18–100%). Five ulcers healed within 12 weeks and four on follow-up within 20 weeks.

As shown in Table 3 a further five patients experienced a substantial reduction in ulcer size of 60–88% by 12 weeks of HOCl treatment, but for social and other reasons were not followed up. Thus, in 14 of the 20 patients (70%), a substantial benefit in terms of healed or healing ulcers was obtained.

As shown in Table 4, six out of 20 patients (30%) treated with HOCl failed to achieve either healing or a substantial reduction in ulcer size. The clinical background of these six patients was, overall, considerably poorer than those whose ulcers had responded. Of particular interest was that five (83%) had hypertension, compared with only four (28%) of the 14 patients whose ulcers had responded (Tables 2 and 3). Particular attention to the management of hypertension in these patients may therefore be appropriate as some antihypertensive drugs, particularly β -blockers, can affect the peripheral microcirculation.

Patient acceptability of HOCl treatment

Fourteen of the 20 patients who initially had pain in the modified McGill questionnaire at levels three to five rapidly reduced these pain levels to nil to one. The remaining six patients did not have pain at the start of treatment above level one, that is minimal discomfort.

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Table 2. Nine patients who failed to achieve ≥44% reduction in ulcer size after three weeks' standard treatment, but who healed after subsequent treatment with HOCl for 12 weeks

Initials/ age (yrs)	Size of ulcer on admission (cm ²)	Duration of ulcer (months)	Reduction of ulcer size after three weeks' standard treatment	Reduction of ulcer size after HOCl washes	
				At 5 weeks	Time to complete healing
BF/82	3.6	9	15%	50%	19 weeks
BC/68	7.0	7	2%	44%	15 weeks
DH/75	4.2	7	31%	75%	8 weeks
MEH/70	13.5	14	34%	85%	9 weeks
KC2/29 M	10.5	15	22%	65%	9 weeks
VB/70	8.3	6	24%	100%	4 weeks
MHO/58	5.0	15	0%	51%	9 weeks
MT/85	5.8	5	0%	38%	16 weeks
JS/79	6.0	12	23%	18%	20 weeks

M = male

Adverse effects of HOCl washes

There was no discomfort from the HOCl washes, except for one patient who, after four weeks' treatment, developed a low-grade eczema, suspected to be caused by the HOCl. The eczema progressed and, although the ulcer size had reduced by 46% at seven weeks, the HOCl treatment was discontinued. The patient's (BF, Table 2) eczema resolved and the ulcer healed at 19 weeks.

There were no other adverse effects. Of the 20 patients who received the washes, the first 14 had their blood biochemistry and haematological assessments repeated at the end of treatment. There were no abnormal changes.

Discussion

Despite marked improvements in the past 15 years there remains a need to further enhance treatment of venous leg ulcers, particularly for patients with chronic and recurrent ulcers, especially over 5cm² and older than six months. We therefore investigated whether washing ulcers with HOCl would bring an improvement to current practice in patients with venous ulcers. The statistical method used to evaluate the results is based on Phillips et al.⁸ and Margolis et al.⁹ The former showed that the baseline ulcer area and ulcer duration are significant predictors of 100% healing and that the percentage healing of the ulcer area at week 3 of treatment is a good predictor of subsequent complete healing, with only 22% achieving healing if they failed to reduce their ulcer size by 44%. Margolis et al. have developed several models to predict the probability of healing and have collected a valuable database for this pur-

pose.⁹ For example, a score of one point is for a wound ≥5cm² while a wound older than six months also scores one point. A combined score of two resulted in healing occurring in only 13% on average (range 6–24%) of patients treated for 24 weeks. Patients with a total score of one achieved healing in 65% of cases (range 54–76%) and with a score of zero 93% healed (range 87–97%).

It could be questioned whether these American findings are applicable to the UK in view of the differences in treatment in the two countries, particularly the Americans' use of Unna's boot. However, a comparative study in Oxford and Philadelphia, USA, has shown that, with either method of compression, very similar healing rates were achieved after 12 weeks and one year of treatment.⁶ A UK study involving 297 patients confirmed the Margolis model's predictive value, with only two (6.7%) of the 30 patients with scores of two achieving ulcer healing within 24 weeks of treatment.¹¹

As it was impossible to do a RCT and given the need to complete a study in two years when three authors were due to retire, we used these published prognostic factors to design this study. Thus, failure to achieve a 44% reduction in wound size at three weeks was used to select patients with only a 22% likelihood of healing with standard compression.

We used the scoring system of Margolis et al.⁸ to allow assessment of the efficacy of treatment to be shortened to 12 weeks' treatment and eight weeks' follow-up. Thus, using Tables 2–4, six (43%) out of 14 patients with a score of two healed, which is outside the 95% confidence interval, indicating that p<0.05. This equated to a relative probability of being com-

Table 3. Five patients who failed to achieve $\geq 44\%$ reduction in ulcer size with the initial standard treatment, but achieved $>60\%$ reduction after HOCl wash plus standard treatment

Initials/ age (yrs)	Size of ulcer on admission (cm ²)	Duration of ulcer (months/years)	Ulcer size after three weeks' standard treatment	Reduction of ulcer size after HOCl washes	
				At 5 weeks	At 12 weeks
VW/85	71.6	5 years	Increased by 5.4%	49%	71%
BW/78	2.0	15 months	Reduced by 18.0%	50%	88%
KCI/29 M	13.5	15 months	Reduced by 22.0%	30%	60%
SB/71	47.1	18 months	Reduced by 1.2%	77%	83%
JD/69	20.0	5 months	Reduced by 6.2%	74%	70%

M = male

Table 4. Six patients who failed to achieve $\geq 44\%$ reduction in ulcer size with the initial standard treatment and who failed to achieve $\geq 60\%$ reduction after 12 weeks' additional treatment with HOCl

Initials/ age (yrs)	Size of ulcer on admission (cm ²)	Duration of ulcer (months)	Ulcer size after three weeks' standard treatment	Reduction of ulcer after HOCl washes	
				At 5 weeks	At 12 weeks
JC/85	25.5	16	Reduced by 35%	23%	21%
PE/48 M	85.9	12	Increased by 34%	33%	41%
GK/72	4.5	3	Reduced by 6.3%	17%	39%
MA/71	9.8	23	Reduced by 12.8%	13%	13%
KH/83	5.6	7	No reduction	13%	29%
PG/57 M	6.8	12	No reduction	12%	27%

M = male

pletely healed on HOCl treatment compared with Margolis et al.'s results, cohort of (6/14) compared with 9/67 = 3.19 (95% confidence interval, range 1.35–7.52). This suggests that patients were three times more likely to heal with the new treatment than the comparator treatment in the paper by Margolis et al.⁹ A test comparing the proportions, Yates-corrected chi-square test, yields a p value of 0.028.

We are well aware of the pitfalls of using historical controls and must be cautious in this statistical interpretation, but note that it is supported by the overall clinical benefit achieved by 70% of the 20 patients in this study.

Study limitations are that the HOCl solution is not yet widely available.

Conclusion

Use of HOCl to wash venous leg ulcers for 12 weeks, alongside standard compression bandaging, materially improved treatment outcomes for large chronic venous leg ulcers. Forty-five per cent (nine) achieved

complete healing and 25% (five) a substantial reduction in ulcer size, giving an overall beneficial result in 70% (14) of the 20 patients. Pain was immediately relieved in all patients and remained absent during treatment and follow-up.

This study's main aim was to examine the role of HOCl in the treatment of difficult-to-cure chronic venous ulcers. Its success in this respect raises the following questions for its further potential use:

- In the treatment of less severe venous ulcers, would it be possible to reduce the time to achieve complete healing from one year to 20 weeks or even less?
- Would its marked antibacterial action be particularly important in the treatment of the more difficult-to-heal diabetic ulcers where bacterial infection can lead to amputation or even be life-threatening?

It might be appropriate in these latter patients to consider increasing the HOCl washes to three or five times per week in the first few weeks, as in our preliminary studies.³ This would require further studies in this field. ■

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