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Evaluating the therapeutic efficacy of ozone liquid dressing in healing wounds associated with bullous pemphigoid

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Bullous pemphigoid (BP) is a chronic autoimmune condition characterized by painful blistering wounds. While effective, conventional treatments often have significant side effects. This study evaluates the therapeutic efficacy of Ozone Liquid Dressing (OLD), an innovative adjunct treatment, in enhancing wound healing, reducing infection rates, and alleviating pain associated with BP. A total of 120 BP patients were assigned to either an observation group (standard care + OLD) or a control group (standard care alone). The dressing was applied daily until wound healing, or the two-week observation period concluded. Efficacy was measured by healing rates, infection reduction (assessed by positive bacterial cultures in wound exudates), and pain levels (assessed by the Numeric Rating Scale, NRS). Statistical analyses were performed using SPSS software, employing t-tests and Chi-square tests as appropriate. The observation group showed significantly higher complete healing rates (61.70% vs. 38.33%, $p < 0.05$) compared to the control group. In terms of overall efficacy, the observation group achieved 91.70%, compared to the control group's 80.00% ($p = 0.116$). A marked reduction in positive bacterial cultures was observed in the observation group, beginning on day 3 ($p < 0.01$), and pain scores decreased significantly by day 10 ($p < 0.001$). OLD significantly enhances wound healing and reduces pain in BP patients, demonstrating clinical potential. Further studies are necessary to confirm the long-term benefits and clinical applicability of OLD in managing BP wounds.

Keywords Bullous Pemphigoid, Ozone liquid dressing, Wound Healing, Infection control, Pain Management

Bullous pemphigoid (BP) is a chronic autoimmune subepidermal blistering condition primarily impacting the elderly, marked by the occurrence of huge, tight blisters on either normal or erythematous skin[1]. Pathophysiology involves autoantibodies against hemidesmosomal proteins, triggering inflammation and blister formation. BP severely impacts quality of life due to pruritus and the risk of infection and presents a therapeutic challenge due to its chronic and recurrent nature. Current treatments primarily rely on systemic corticosteroids and immunosuppressants to reduce inflammation and autoantibody production[2,3]. Nonetheless, these treatments entail numerous potential adverse effects, especially in the elderly, including but not limited to osteoporosis, hypertension, diabetes, and heightened vulnerability to infections. However, these therapies are associated with significant adverse effects, particularly in elderly patients, including osteoporosis, hypertension, diabetes, and increased infection risk. Although topical therapies pose fewer systemic risks, their efficacy in severe cases or widespread lesions is limited, and long-term management is hindered by concerns of cumulative toxicity and drug resistance[4,5].

In this context, Ozone Liquid Dressing (OLD) seems as a possible supplementary treatment. Ozone, a triatomic molecule composed of three oxygen atoms, is acknowledged for its powerful antibacterial characteristics, which can inactivate bacteria, viruses, fungus, and protozoa[6,7]. OLD combines these benefits with the convenience of a liquid dressing, potentially improving clinical outcomes in BP wounds[8,9]. Its antibacterial action minimizes secondary infections, while its immunomodulatory effects may alleviate BP's characteristic inflammatory response and facilitate wound repair[10,11].

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While ozone therapy has shown promise in other wound types, its efficacy in BP-related wounds remains underexplored[12–14]. This paper seeks to systematically assess the therapeutic efficacy of OLD in the healing of wounds related to BP, examining its potential to overcome the limitations of existing treatments while providing a safer, more tolerable, and potentially more effective therapeutic option. This study aims to elucidate the distinct benefits and therapeutic effects of OLD in relation to BP, thereby providing significant insights into the management of this complex dermatological condition, which may transform existing treatment strategies and enhance the quality of life for affected individuals.

Materials and methods

Study design

A complete retrospective study was undertaken at our institution to evaluate the therapeutic efficacy of OLD in treating wounds related to BP. The inquiry extended from June 2021 to January 2024. The study comprised 120 patients, categorized into two separate groups for comparative evaluation. The observation group consisted of 60 patients who had a combination therapy of standard treatment methods and OLD. Conversely, the control group comprised 60 patients treated within the same period, who received just conventional therapies without the addition of OLD. This parallel group was created to function as a benchmark, facilitating a comprehensive comparison between the conventional treatment regimen and the novel strategy utilizing ozone therapy. Informed consent was obtained from all subjects and/or their legal guardian(s). The research design, objectives, and procedures were meticulously reviewed and approved by the Ethics Committee of our institution. All methods were performed in accordance with the relevant guidelines and regulations. The design, performance, and reporting of this study strictly followed the ethical principles for medical research involving human subjects outlined in the Declaration of Helsinki. Data was handled confidentially, and all personal identifiers were removed prior to analysis to protect participant privacy.

Inclusion and exclusion criteria

Inclusion criteria

(1) Clinically and histopathologically verified diagnosis of BP. Ensures an accurate and standardized BP patient population.

(2) Presence of active lesions related to BP at the time of participation. Guarantees that any observed therapeutic effects directly relate to active wound sites.

(3) A minimum of one month of conventional therapy, including corticosteroids or immunosuppressive drugs, before the research begins, ensuring disease stability at baseline. Establishes a controlled baseline, minimizing variability in disease progression caused by recent medication changes.

(4) Written informed consent provided by participants, confirming their understanding of the study's methods and the use of their data. Complies with ethical guidelines and guarantees patient awareness and voluntary participation.

Exclusion criteria

(1) Concurrent dermatological illnesses, such as psoriasis, eczema, or other blistering diseases that may disrupt wound healing or complicate the evaluation of BP lesions. These could confound wound-healing assessments specific to BP and limit the study's internal validity.

(2) Severe systemic diseases or conditions that may hinder wound healing or increase the likelihood of adverse events, such as: Uncontrolled diabetes, Congestive heart failure, Chronic renal failure. Such comorbidities may independently impair wound healing or elevate risks, potentially confounding outcome measures.

(3) Documented allergy or hypersensitivity to ozone or any components of the OLD, to prevent adverse reactions and ensure participant safety. Ensures patient safety by avoiding known adverse reactions to the experimental dressing.

Preparation of ozone liquid dressing

A unique method for creating an antibacterial, antiviral, and hygroscopic ozone oil hydrogel composite dressing was created to overcome the limitations of standard dry dressings in wound healing, including sluggish healing rates, discomfort, and secondary injury during dressing changes. The preparatory procedure encompassed numerous essential steps. Polyvinyl alcohol (PVA) was initially dissolved in deionized water heated at 92–96 °C, including 8–12 g of PVA per 100 ml of water. The solution was agitated for 2–5 h and subsequently cooled to room temperature to yield a PVA solution. A graphene oxide solution at a concentration of 4 mg/ml was subsequently added to the PVA solution in volume ratios from 1:0.5 to 2, and the combination was stirred at room temperature for 12 to 24 h to get the reaction mixture. The mixture was subsequently moved to a glass container, sealed, and subjected to a freeze-thaw cycle at -15 to -20 °C, followed by thawing at ambient temperature, to produce the composite hydrogel. The posterior layer of the composite hydrogel was covered with silica gel and subjected to dehydration and aging at 60–70 °C for 3–5 h. Ozone oil was subsequently administered to the inner layer of the composite hydrogel. The hydrogel's posterior layer was subjected to vacuum treatment prior to sealing and resting, with the volume ratio of ozone oil to PVA solution established between 1:3 and 6, resulting in the creation of the antibacterial, antiviral, and hygroscopic ozone oil hydrogel composite dressing.

Intervention methodology

The control group in this study got normal treatment regimens without any interventions. The observation group received OLD administered to the wound sites, in addition to normal treatment. The dressing was used daily

until the wound healed or until the conclusion of the two-week observation period. All patients, irrespective of group assignment, adhered to a uniform treatment protocol throughout the entire trial period.

Data collection

The study monitored and documented the healing duration, infection rates, and pain levels in the wounds of both cohorts. Wound healing was evaluated according to criteria indicating that erosive areas were fully re-epithelialized, desiccated, and devoid of exudate. A subgroup within each cohort, including 3 to 5 erosive wounds, was deemed totally healed only when all designated wounds satisfied the established healing criteria. Wound site infections were detected via the culture of exudates. Pain levels were objectively evaluated using the Numeric Rating Scale (NRS)[15], which spans from 0 to 10, with 0 denoting no pain and 10 signifying the most intense pain. The pain scale was delineated as follows: 0 for no pain, 1–3 for mild pain, 4–6 for moderate pain, and 7–10 for severe pain, to enhance comprehension of patients' pain experiences during therapy and recovery.

Statistical analysis

The statistical analysis was performed using SPSS software (Version 27.0) for thorough data evaluation. Data were first classified into quantitative or categorical kinds, thereafter undergoing normality tests to ascertain their distribution features. For quantitative variables following a normal distribution, group differences were evaluated using independent samples t-tests, with results shown as mean ± standard deviation. When quantitative data exhibited non-normal distribution, the median and interquartile ranges (M[P25, P75]3.1) were reported, and group differences were analyzed using the Mann-Whitney U test. Categorical data were represented as counts and percentages, and Chi-square ($\chi 2$) tests were employed to examine the correlations or differences among these variables. The analysis utilized two-tailed hypotheses, establishing a p-value threshold of less than 0.05 to ascertain statistical significance.

Results

Clinical characteristics of wound lesions in the observation and control groups

In the observation group, the wound areas ranged from 5 to 9 cm^2, with an average size of 6.5 ± 1.8 cm^2. The distribution of these lesions included 5 on the head, face, and neck regions, 22 on the trunk, and 33 on the extremities. Similarly, in the control group, wound areas also ranged from 5 to 9 cm^2, with an average size of 6.3 ± 1.7 cm^2. Statistical analysis revealed no significant differences between the two groups in terms of wound size and distribution ($P > 0.05$), indicating that the baseline characteristics were comparable.

Comparative outcomes of wound healing using ozone liquid dressing

The integration of OLD into the normal therapy protocol (observation group) resulted in a significantly elevated complete healing rate, with 61.70% of patients attaining full recovery, compared to 38.33% in the standard treatment-only group (control group). The differentiation was statistically significant, indicated by a chi-square value of 5.63 and a p-value below 0.05. The observation group attained an overall treatment efficacy rate of 91.70%, surpassing the 80.00% rate recorded in the control group, when considering full healing and significant improvement. Notwithstanding the apparent enhancement in efficacy, the statistical analysis revealed that the difference was not significant, evidenced by a chi-square value of 2.48 and a p-value of 0.116. The results indicate that the supplementary use of OLD alongside conventional therapy may significantly improve the probability of complete wound healing. Although the overall efficacy improvement did not reach statistical significance, the results suggest a positive trend in favor of OLD for wound management (Table 1).

Impact of ozone therapy on bacterial cultures in wound healing

The study indicated a significant reduction in positive bacterial cultures over time in the observation group, which received both conventional treatment and OLD, while evaluating the microbial component of wound healing. At the outset, both the observation and control groups demonstrated comparable rates of positive bacterial cultures prior to treatment. Nonetheless, a notable discrepancy in bacterial reduction was seen by day 3 post-treatment, with the observation group exhibiting a considerable decline in positive cultures. This pattern persisted on days 5, 7, and 10, during which the observation group exhibited a near-complete eradication of positive bacterial cultures, whereas the control group, receiving solely conventional treatment, sustained elevated levels of bacterial presence. The statistical analysis, as evidenced by chi-square values, revealed a significant difference in the reduction of positive bacterial cultures from day 3 post-treatment, with p-values below the 0.01 level. The results indicate that the supplementary application of OLD in wound treatment not only enhances

Group	Sample Size	Healed	Marked Improvement	No Effect	Efficacy Rate (%)	Cure Rate (%)
Observation	60	37	18	5	91.70	61.70
Control	60	23	25	12	80.00	38.33
Chi-square χ^2	-	-	-	-	2.48	5.63
p-value	-	-	-	-	0.116	< 0.05

Table 1. Comparative analysis of Wound Healing over Time between Observation and Control Groups. Note: The efficacy rate is defined as the sum of patients who experienced healing and marked improvement. The cure rate is the percentage of patients who were completely healed.

Group	Sample Size	Pre-treatment	Day 3 post-treatment	Day 5 post-treatment	Day 7 post-treatment	Day 10 post-treatment
Observation	60	46	17	7	3	0
Control	60	45	39	33	25	12
Chi-square χ^2	-	0.00	14.76	23.43	20.54	11.2
p-value	-	1.00	< 0.01	< 0.01	< 0.01	< 0.01

Table 2. Comparison of positive bacterial cultures in Wound exudates pre- and Post-treatment. Note: The Chi-square statistic and p-value are provided for days 3, 5, 7, and 10 post-treatments. A p-value of less than 0.01 indicates a statistically significant difference between the observation and control groups at the respective time points.

Group	Sample Size	Pre-treatment	Day 3 post-treatment	Day 7 post-treatment	Day 10 Post-treatment
Observation	60	8.35 ± 0.66	4.06 ± 0.62	1.69 ± 0.69	0.60 ± 0.64
Control	60	8.45 ± 0.64	7.69 ± 0.68	6.14 ± 1.07	4.28 ± 0.70
t-statistic	-	0.796	31.279	27.866	30.991
p-value	-	0.428	< 0.001	< 0.001	< 0.001

Table 3. Comparative analysis of Numeric Rating Scale before and after treatment (Mean ± SD).

bacterial elimination but may also foster a more sterile wound environment, hence potentially diminishing the risk of infection and facilitating expedited wound healing (Table 2).

Impact of ozone therapy on pain reduction in wound healing

The study's examination of pain alleviation, measured by the NRS, highlights the enhanced therapeutic impact of integrating OLD into the conventional wound care protocol. Although baseline pain levels were similar in the observation and control groups, the intervention group exhibited a marked and substantial reduction in pain scores following treatment. By day three, the observation group's average pain score had decreased by 50%, and by day ten, it was reduced to less than one-seventh of the pre-treatment level. In contrast, the control group had a less significant decrease in pain, sustaining elevated mean scores during the treatment period. The statistical validity of these findings is supported by strong t-statistics and p-values, demonstrating a highly significant difference in pain score reduction favoring the observation group from day 3 onward ($p < 0.001$). These findings indicate a significant potential of OLD in improving patient comfort and alleviating the subjective perception of pain, a crucial element of the wound healing process (Table 3).

Post-hoc power analysis

A post-hoc power analysis using the weighted effect size method was conducted to evaluate the robustness of our findings. The analysis indicated high overall statistical power (96.7%), with strong effects observed in bacterial culture reduction (medium effect size, Cohen's w = 0.35) and pain relief (very large effect size, Cohen's d = 2.5), yielding powers of 91% and 100%, respectively. Although the effect size for wound healing (Cohen's w = 0.2166) was smaller, the substantial effects in bacterial reduction and pain relief significantly contributed to the study's high power. These results highlight the therapeutic potential of OLD in improving bacterial clearance, alleviating pain, and enhancing wound healing, thereby supporting its clinical application in managing BP.

Discussion

BP is an autoimmune blistering disorder primarily affecting older adults, causing chronic, painful, and non-healing wounds[7,16]. Conventional treatments, such as corticosteroids and immunosuppressive agents, are associated with significant adverse effects and slow wound healing[17]. This study explores the potential of OLD as a novel adjunctive therapy to enhance wound healing, reduce infection risk, and improve patient comfort in BP management[18]. This study introduces OLD as an adjunctive treatment for BP wounds, addressing key limitations of conventional therapies[19,20]. OLD combines ozone's antimicrobial, anti-inflammatory, and oxygenating properties in a composite hydrogel, potentially accelerating wound healing, reducing infection risk, and alleviating pain[21]. BP, characterized by chronic, non-healing blisters, poses significant challenges due to slow recovery, high infection rates, and adverse effects from systemic treatments[22,23]. This research demonstrates that OLD significantly improves healing rates, decreases bacterial colonization, and enhances patient comfort. Its non-invasive, cost-effective, and easy-to-administer nature positions OLD as a promising addition to BP wound care, offering a novel, evidence-based alternative to traditional therapies and improving clinical outcomes for patients. Given its ease of use and favorable safety profile, OLD could be integrated into routine clinical practice, particularly for patients who experience limited response to conventional therapies. Moreover, its potential for use in outpatient settings, coupled with its relatively low cost, makes it an attractive option for widespread adoption in both resource-limited and well-resourced healthcare environments. However, additional studies are required to standardize application protocols and further assess its long-term benefits in diverse patient populations.

The significant reduction in positive bacterial cultures in the observation group corresponds with the established antibacterial capabilities of ozone. The swift decrease in bacterial burden following treatment with OLD indicates that ozone may effectively permeate wound exudates, eradicating germs that could hinder wound healing or result in infections[24]. The antibacterial action, in conjunction with the conventional treatment, seems to produce a synergistic effect that diminishes the quantity of pathogenic or opportunistic microorganisms. The control group, dependent exclusively on conventional wound care, demonstrated delayed and less effective microbial clearance, underscoring the potential shortcomings of routine treatment in swiftly mitigating bacterial contamination[25,26]. The persistence of bacteria in wound exudates can impede wound closure and elevate the risk of chronic wound conditions. Alleviation of pain is an essential aspect of wound management, influencing patient adherence and overall quality of life. The observation group's notable reduction in NRS ratings following treatment with OLD signifies an effective analgesic advantage. The analgesic effect may result from ozone's anti-inflammatory capabilities, which can alleviate the inflammatory response that frequently intensifies pain at wound sites[27,28]. Furthermore, the decrease in microbial load may lower the bioburden that might provoke inflammatory cytokines, hence exacerbating pain. The t-statistical analysis confirms that the clinical improvements obtained with OLD are significantly superior to those attained with conventional treatment alone[29,30]. Nonetheless, the lack of a substantial change in the total treatment efficacy rate, despite the observable trend, indicates that although OLD may improve healing quality, its effect on the healing rate requires additional investigation.

The mechanisms behind the therapeutic effects of OLD are probably multifaceted. Ozone may modulate inflammation in BP lesions through several molecular pathways. By inducing mild oxidative stress, ozone activates antioxidant defenses, reducing excessive ROS and suppressing pro-inflammatory cytokines like TNF-α and IL-6 while increasing anti-inflammatory IL-10. This rebalancing of cytokine profiles helps restore immune homeostasis and promotes a transition from inflammation to tissue repair. Ozone also partially inhibits NF-κB activity, reducing inflammatory mediator production[31]. Additionally, by enhancing local oxygen availability, ozone stimulates fibroblast proliferation, migration, and extracellular matrix production, including increased collagen synthesis through TGF-β signaling[32]. This supports granulation tissue formation, structural integrity, and re-epithelialization. These combined effects suggest that OLD not only targets bacterial load but also fine-tunes inflammation and fibroblast activity, fostering an environment conducive to wound healing and tissue regeneration. Understanding these mechanisms may help optimize OLD's clinical application and improve patient outcomes in BP wound care. In our investigation, the observation group, administered OLD, had a superior overall healing rate relative to the control group. Notably, the control group demonstrated a greater proportion of wounds categorized as significantly improved, despite a lower rate of complete healing. This observation necessitates a thorough discussion. It indicates that although OLD expedites the journey to full healing, traditional treatment may provide more reliable results in attaining partial, albeit not total, recovery of the wound condition. This may be due to the antibacterial qualities of the ozone dressing, which could more effectively reduce impediments to complete healing, such as bacterial load, compared to conventional treatments alone. This difference outcome underscores the potential benefit of incorporating ozone therapy as a supplemental treatment, hence strengthening specific therapeutic elements of wound care in BP[33].

This study investigating the efficacy of OLD for BP wounds has several limitations. First, as a retrospective study, it is limited by the available clinical data, which may restrict the generalizability of the findings. Although the sample size was relatively small, post-hoc power analysis confirmed that it was sufficient to detect statistically significant differences. The absence of stratification based on disease severity could have influenced the treatment outcomes. A placebo-controlled design was not feasible, as treatment plans were established prior to patient enrollment, limiting our ability to fully attribute improvements in wound healing to the effects of OLD. Additionally, the study focused on short-term outcomes without long-term follow-up, leaving the sustained efficacy and potential late-onset adverse effects of OLD unexplored, which may lead to an underestimation of long-term complications. Finally, although a reduction in bacterial load in wound exudates was observed, these cultures may not accurately reflect deeper tissue infections, as they are likely to indicate surface contamination rather than true infections. Future research on OLD should prioritize large-scale, prospective randomized controlled trials (RCTs) with stratification based on BP disease severity to validate its therapeutic efficacy. Long-term follow-up studies are necessary to assess the durability of OLD's effects and monitor for delayed adverse reactions. Additionally, investigating the biological mechanisms, including cellular and immune responses, as well as dose-response relationships, will optimize its clinical use. Comparative studies with established BP treatments and exploration of synergistic effects with other therapies are essential to expand its clinical applicability. A comprehensive cost-effectiveness analysis will further support its potential as a viable, non-invasive adjunct in wound healing.

Conclusions

This retrospective study highlights the potential of OLD as an adjunctive therapy for BP wounds, demonstrating improvements in wound healing, infection control, and pain relief. These preliminary findings suggest that OLD could offer significant benefits in clinical practice. However, further prospective studies are necessary to confirm its long-term efficacy, assess sustained outcomes, and optimize its integration into treatment protocols.

Data availability

The datasets used and/or analyzed during the present study are available from the corresponding author upon reasonable request.

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Author contributions

Conceptualization: Li Li.Data curation: A'chong Feng.Formal analysis: Li Li, Jianyun Lu.Methodology: Hongye Liu.Resources: Li Li.Software: Wenli Xue.Writing – original draft: Li Li.Writing – review & editing: Hongzhou Cui.

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Declarations

Ethics approval and consent to participate

All methods employed in this study adhered to relevant guidelines and regulations. The study received approval from the Ethics Committee of The First Hospital of Shanxi Medical University (20210527). Written informed consent was obtained from all subjects participating in the study.

Consent for publication

Informed consent was obtained from all subjects and/or their legal guardian(s). Patients and/or families in the study provided consent for the publication of their data.

Competing interests

The authors declare no competing interests.

Additional information

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