Efficacy of a Solution Composed by Verbascoside, Polyvinylpyrrolidone (PVP) and Sodium Hyaluronate in the Treatment of Chemotherapy-induced Oral Mucositis in Children With Acute Lymphoblastic Leukemia

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Summary: The aim of this study was to assess the efficacy of a solution composed by verbascoside, polyvinylpyrrolidone, and sodium hyaluronate (Mucosyte) in the treatment of chemotherapy-induced oral mucositi (OM). Patients between 5 and 18 years receiving chemotherapy for acute lymphoblastic leukemia and with OM grade 1 or 2 were randomized in group A (treated with Mucosyte, 3 mouthwashes/d per 8 d) and group B (treated with placebo, ie, an inert water-based solution, 3 mouthwashes/d per 8 d). The OM scoring was performed at day 1 (diagnosis of OM-T0), after 3 days of treatment (T1), and at day 8 (T2). Pain was evaluated through the visual analog scale with the same timing of OM measurement. A total of 56 patients were included (28 patients per group). Group A experienced a statistically significant decline of OM at T2 ($P = 0.0038$); a statistically significant difference in pain reduction between 2 groups both at T1 and at T2 ($P < 0.005$) was observed. The use of Mucosyte mouthwashes in children with chemotherapy-induced OM may be recommended as supportive therapy.

Key Words: mucositis, child, chemotherapy, verbascoside

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Oral mucositis (OM) is a common side effect of chemotherapy, especially in case of hematopoietic stem cell transplantation, due to the high-dose myeloablative chemotherapy used for conditioning regimens.[1] The incidence of OM in the pediatric population varies from 50% up to 80%.[2,3] Mucositis significantly affects the quality of life in terms of pain, ability to eat, swallow, and talk; indeed, symptoms are often of such severity as to require an interruption or curtailment of therapy. In the presence of neutropenia, mucositis predisposes to septicemia, bacteremia, and fungemia. Moreover, the presence of OM, lengthening the hospitalization timing, is an important driver of health care costs.[4] Treatment guidelines for OM were issued in 2004 and were recently updated by the Multinational Association of Supportive Care in Cancer and International Society for Oral Oncology (MASCC/ISOO). Current management of OM might comprise growth factors and cytokines, anti-inflammatory agents, anesthetics, analgesics, antimicrobial and coating agents, cryotherapy, and mucosal protectants.[5–7] Although several preventive and therapeutic approaches have been evaluated, no single agent has been found to be superior. Given the side effects of pharmaceutical agents, complementary therapies are increasingly being used all over the world to especially support OM-low grade. In recent years, more attention has been focused on the role of natural drugs.[8] These "natural substances" are low-molecular weight organic compounds found in plants that activate a defence system against physiological and/or environmental stress.[9–11] Such compounds have antioxidant, antimicrobial, and immunomodulatory properties and derive from secondary metabolites, which belong mainly to the category of terpenes, terpenoids, alkaloids, phenols, and flavonoids.[12–14] Polyphenols compound are secondary metabolites widely distributed in the plant kingdom, with many biological properties, antiallergic, antiatherogenic, anti-inflammatory, antimicrobial, and antioxidant.[15] Of particular interest, in recent years, are the phenylpropanoid glycosides (also synonymous with phenylethanoid glycosides) which are water-soluble derivatives of phenylpropanoids, a large group of natural polyphenols.[16] Verbascoside, a polyphenol present in many plants used for food, flavouring, and medicines like olive and mullein, shows the highest scavenger activity among phenylpropanoid glycosides tested, as well as a high antioxidant power, even in comparison with other natural phenolic compounds.[17,18] In fact, it inhibits and blocks proinflammatory enzymes activity, especially of inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2).[19]

Recent studies have indicated that the fundamental mechanisms involved in the pathogenesis of mucositis are much more complex than direct damage to epithelium alone. Chemotherapy induces cellular damage resulting in death of the basal epithelial cells. The generation of reactive oxygen species is believed to exert a role in the initiation of mucosal injury.[6]

The aim of this study was to assess the efficacy of a solution composed by verbascoside, polyvinylpyrrolidone (PVP), and sodium hyaluronate (Mucosyte; Biopharm, Milan, Italy), respect of placebo, in the treatment of chemotherapy-induced OM.

MATERIALS AND METHODS

Sample Selection

The study was carried out at the Pediatric Dentistry Department of the University of Brescia and at the

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Paediatric Oncology Unit of Spedali Civili of Brescia (Italy). Consecutive patients receiving chemotherapy for acute lymphoblastic leukemia (ALL) (elaborate on the 2009 standard protocol) and with OM grade 1 or 2 were considered eligible for this study.

Enrolment criteria were: patients aged between 5 and 18 years with ALL undergoing any 1 of the phases of the chemotherapy protocol and with OM grade 1 or 2. Exclusion criteria were: OM grade >2, other hematologic malignancies than ALL, hypersensitivity or allergy to any of the components included in the study; radiotherapy; antibiotic and antifungal drugs assumption. Age, sex, oral hygiene grade, and absolute neutrophil count (ANC) were recorded for each patient.

Study Design

This study was designed as a double-blinded placebo-controlled study. Patients were randomized by the computer code in 2 groups: group A treated with Mucosyte (Biopharm) rinses and group B treated with placebo (ie, an inert water-based solution). The 2 products were identical regarding packaging and texture, identified only by a color label. Patients were instructed to rinse with 15 mL of solution (Mucosyte or placebo) for 2 minutes 3 times per day for 8 days.

Randomization was performed using an automatically generated list in a 1:1 block size for 2 patients. Patients included in the study were randomly assigned to 1 of the 2 treatments. Patients received a number after inclusion, corresponding to a color-coded mouth rinse (red for Mucosyte and blue for placebo). The list and the colored bottles were provided by the statistic while the clinical researchers made the assignation of numbers in consecutive order. Color codes were not opened until the end of the study. Both patients and researchers were blinded throughout the study.

OM Scoring

OM grade was scored by the same 2 pediatric dentists blind to the randomized allocation, according to the Common Toxicity Criteria Scale of the WHO. Lesions were classified as grade 0 = none; grade 1 = soreness and erythema; grade 2 = erythema and ulcers without difficulties in swallowing solid food; grade 3 = ulcers requiring only a liquid diet, and grade 4 = introduction of parental nutrition.

Oral examination and OM scoring were performed at day 1 (diagnosis of OM) (T0), after 3 days of treatment (T1) and at day 8 (T2).

PAIN Scoring

Pain was evaluated through the visual analog scale (VAS) at the same timing of OM scoring. In this system, 1 indicates no pain and 10 indicates severe pain; patients were asked to select a number from 1 to 10 on a ruler with drawn faces to express the intensity of their pain (Wong Baker face scale). Request of additional analgesia, which means the number of times that children required pharmacological support, was also recorded.

Oral Hygiene

Clinical assessment of oral hygiene status was performed according to criteria of simplified oral hygiene index (OHI-s) by Greene and Vermillion[20] with a mouth mirror and explorer.

Statistical Analysis

Statistical analysis was descriptive, including mean, SD, and percentiles for variables as sex, age, and localization.

To compare the 2 groups, we analyzed the data on sex and disease as frequencies and percentages. Concordance or differences in the frequency distribution between the 2 groups were tested using Student t test. A level of significance of 5% was used and data were analyzed using Stata software for Mac. The differences in decline of OM and VAS score were analysed using Wilcoxon rank sum test. The difference of the mean of the OHI-s and ANC at T0 and T2 was evaluated using 2 ways analysis of variance.

Taking the hypothesis that there is a success percentage of 80% at day 8 for the group treated by Mucosyte rinses and 40% for control group, the minimum number of patients for the study, assuming $\alpha = 0.05$ and $\beta = 0.20$ (study power = 80%), has been calculated to be 55 (at least 23 per group).

Ethical Considerations

The study was planned and carried out in compliance with the Declaration of Helsinki and Good Clinical Practice. All patients and their caregivers were informed about the research and signed an Institutional review board-approved informed consent.

RESULTS

A total of 59 patients were included in the study according to the enrolment criteria. Three were excluded because caregivers did not sign the informed consent. Group A (Mucosyte) was composed by 28 children (19 female and 9 male, mean age 7 ± 1.8) and group B (placebo) by 28 children (15 female and13 male, mean age 8 ± 2.7).

OM Grading

OM appeared at a mean of 6.1 days after chemotherapy (range, 4 to 8 d).

At T0, the median of OM grading was 2 for group A and 2 for group B ($P > 0.05$). At the third day (T1), OM grading median was 1 for group A and 2 for group B ($P = 0.65$); after 8 days (T2), OM grading median resulted 0 in group A and 1 in group B ($P = 0.038$). Group A experienced a statistically significative decline of OM just at T2 ($P = 0.0038$) (Fig. 1).

Pain Evaluation

The medians of VAS at the 3 intervals and P-values obtained by Wilcoxon rank sum test are displayed in Table 1. A statistically significative difference in pain reduction between 2 groups both at T1 and at T2 ($P < 0.05$) was observed. During the study period, children treated by Mucosyte rinses required less additional analgesia (paracetamol, tramadol, or morphine) with respect to those belonging to the control group ($P < 0.05$).

Oral Hygiene and ANC

The mean of OHI-S at T0 and T2 in both groups was not statistically different ($P = 0.15$).

The mean of ANC at T0 and T2 in both groups was not statistically different ($P = 0.63$).

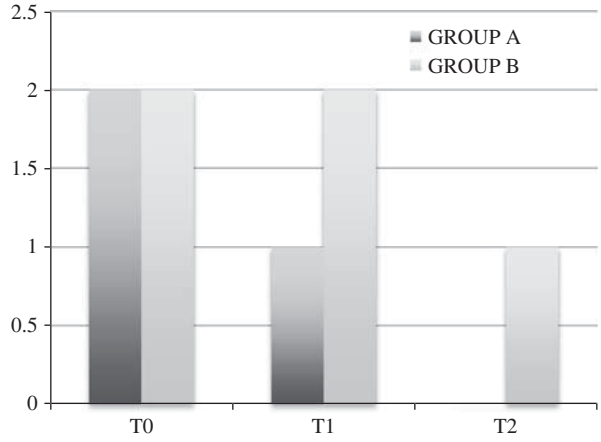

FIGURE 1. Results for decline in oral mucositis grade at T0, T1, and T2 in the group A and B. T0 indicates diagnosis of oral mucositis on day 1; T1, after 3 days of treatment; T2, at day 8.

DISCUSSION

OM can aggravate the pediatric patients' clinical condition and elicit multiple debilitating oral symptoms that irrevocably alter patients' quality of life.[2]

The pathogenesis of chemotherapy-induced OM appears to be related to oxidative stress induced by the treatment. The reactive oxygen species cause direct and indirect damage through transcription factors, such as nuclear factor-kB, that activates iNOS, and the activator protein-1, which induce the production of proinflammatory cytokines, such as tumor necrosis factor and interleukin-6.[21,22]

This pilot study was designed to investigate the efficacy of a solution, containing sodium hyaluronate, PVP, and verbascoside, in chemotherapy-induced OM. Verbascoside is the extract of syring vulgaris and it belongs to the fenil-propanoid family, a new class of anti-inflammatory drugs.

Previous studies have suggested that verbascoside can prevent the oxidative stress as it reduces the production of superoxide radicals and consequently reduces the activity of iNOS and COX-2 [23]. Moreover, it is involved in controlling transforming growth factor beta-activated kinase, a novel protein that mediates proinflammatory signaling through induction of the transcription factor activator protein -1. Verbascoside induces the dose-dependent decrease of the expression of interleukin-8, inhibiting proinflammatory activity of enzymes like COX-2 and iNOS, showing a cortison-like activity.[14,23] Recent studies have demonstrated that sodium hyaluronate accelerates healing[24] and helps to manage pain[25] in patients with OM. Some authors stated that the compound acts as a physical barrier between the oral environment and oral mucosa, inducing also biomolecular and physiological changes in keratinocytes and mesenchimal cells.[26,27]

TABLE 1. Results for Decline in Visual Analog Scale at T0, T1, and T2 in the Group A and in the Group B

	Group A	Group B	W (Wilcoxon test)	*P*
T0	4	4	3805.5	0.90
T1	1	2	3252	0.002*
T2	0	1	3160.5	0.0005*

Medians, Wilcoxon rank sum test and *P*-value. Test: Wilcoxon rank sum test.

T0 indicates diagnosis of oral mucositis on day 1; T1, after 3 days of treatment; T2, at day 8.

*$P < 0.05$.

The severity of OM is mainly determined by the type(s) and dose of cancer therapeutic agents used. The therapeutic trend of combining different chemotherapeutic drugs further intensifies the likelihood of mucositis.[28] To exclude possible confounding factors, we selected patients with ALL, undergoing the same chemotherapy protocol (ALL 2009 standard protocol), which inter alia induces a less deep and shorter aplasia.[29] Instead, we did not consider the treatment stage as a variable (to evaluate the efficacy of the mouthwash) because there are still no studies that correlate OM (in terms of "duration" and "rate of healing") with the stage of treatment that caused it.

Moreover, we took into account only OM grade 1 or 2, usually requiring only topical therapy, not combined with other device or drugs (ie, laser therapy, grow factors, steroids). As regards the diagnosis of OM, a concomitant herpes simplex virus infection—which is a known risk factor for aggravation of the severity of OM[30]—was excluded because all patients underwent an antiviral prophylaxis (5 mg/kg/dose 3 times a day).

The results of this investigation suggest clinical benefits in the use of the tested solution. According to our results, OM significantly reduces in the study group respect to the control group in 8 days. Also the pain, already after 3 days, is significantly decreased both from VAS results and from the request of additional analgesia. The benefit may depend on the synergic action of active components. The mucoadhesive properties of PVP allow the continuous release of sodium hyaluronate, which favors re-epithelization and wound healing, and of verbascoside, which has an anti-inflammatory action.

To exclude other confounding factors, we considered also the oral hygiene grade and the ANC. The grade of oral hygiene can affect the incidence and severity of mucositis. Maintenance of good and intensified oral hygiene is a fundamental component of basic oral care.[31] When oral hygiene is compromised, gingival and mucosal inflammation caused by oral bacteria increases the risk of OM up to bacteremia.[32–34] According to our results, the grade of oral hygiene was not statistically different in the 2 groups. This result was expected due to the strict oral hygiene protocols to which oncohematological patients are subjected according to the international guidelines.[5,6,31]

ANC was recorded because a decrease in the neutrophil count may result in an impaired ability to protect against oral mucosal damage and may affect the proliferation of oral epithelial cells. Neutropenic patients are at increased risk for microbial colonization of damaged mucosal surfaces, resulting in increased proinflammatory cytokines in oral mucosa, which may aggravate OM.[2,35,36]

The ANC was similar in the 2 groups and ranged between $0.2 \times 10(9)$ and $0.5 \times 10(9)$/L. So, even if the patients with OM tended to be neutropenic, these data did not affect the outcome of the study. The results of this pilot study should be interpreted with caution. Even if we tried to exclude confounding factors, one limitation was the small sample size. Within this limit, we can conclude that the use of Mucosyte may lead to improvements in clinical parameters in children with chemotherapy-induced low-grade OM and it may be recommended as supportive therapy.

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