A New Therapeutic Approach to Treat Oral Mucositis Using Specific MMP Blockers in an Osmotically Active Solution

Ravi Shrivastava*, Swity Deshmukh
R&D department, VITROBIO Research Institute, Issoire, France
*Corresponding author: vitrobio@orange.fr

Received December 31, 2012; Revised February 07, 2013; Accepted March 30, 2013

Abstract Mucositis is a consequence of cytostatic effects of anticancer therapy on the fast growing oral mucosal cells. The size of the ulcer increases due to subsequent bacterial and fungal growth, while the leakage of circulating toxins through damaged capillaries surrounding the ulcers causes severe irritation and burning sensation. Proteolytic enzymes, which are known to destroy cellular matrix, inhibit cell regeneration and healing. We tested OROSOL®, an osmotically active hypertonic solution containing specific protease-inhibiting plant procyanidins capable of cleaning the injury, removing the contaminants and stimulating cell growth as a new multiple therapeutic approach for the treatment of mucositis. Out of 69 total patients, 48 were treated with OROSOL® spray, 4-5 times per day for a period of 28 days. 21 patients in the classical treatment group used standard treatments. The grade of overall mucositis, intensity of pain and burning sensation, formation of new ulcers and effect on eating impairment were evaluated before treatment, 30 minutes after first product application and on days 1, 2, 3, 4, 7, 14, 21 and 28. Data were analyzed using SAS 9.1.3, Statistical program. Compared to the classical treatments group, Orosol® group showed significantly higher improvement in pain, burning sensation, eating abilities, grade of infection and overall mucositis. New ulcer formation rate was not affected. Mucositis treatment requires a multiple therapeutic approach of simultaneously eliminating the contaminants and the toxic chemicals from the ulcer as well as creating a favorable ground for healthy cell growth. OROSOL® seems highly effective in achieving these objectives.

Keywords: mucositis, extra cellular matrix (ECM), matrix metalloproteases (MMPs), procyanidins (PCDs), tannins, hypertonic

1. Introduction

Oral mucositis is one of the most debilitating side effects of radiation therapy and various forms of chemotherapy, particularly for head and neck cancers and hematopoietic stem cell transplants. Mucositis has been shown to significantly lengthen hospital stays to manage pain, secondary infections and tube feeding, leading to excessive economic burdens and physical as well as moral suffering for the patient [1].

Oral mucositis usually develops within seven to fourteen days after chemotherapy or radiation therapy is initiated and lasts up to the end of the treatment period. As all the anticancer therapies are directed to stop the growth of cancerous cells, and as none of the currently available anticancer drugs can differentiate between normal and tumor cells, collateral damage to the normal healthy cells cannot be avoided. Oral mucosal cells are one of the fastest growing cells in the body and are therefore particularly affected by all anticancer treatments which block cell growth, leading to the formation of multiple injuries on the oral mucosa. An ideal temperature and availability of nutrients favor the development of microorganisms, so that the ulcer often gets contaminated with bacteria, fungus and even viruses [2]. Depending on the severity of ulceration, infection, and possibilities of food intake, oral mucositis is scored between grade 0 (absent) and grade 4 (maximum).

Although theoretically mucositis should be easy to treat as it simply requires cleaning the ulcers and stimulating the growth of the underlying healthy cells to fill the ulcer, currently there are only symptomatic treatments for mucositis.

As oral mucositis ulcers do not heal easily, they become chronic. All chronic wounds are known to contain excessive quantities of proteolytic enzymes or Matrix MetalloProteases (MMPs) [3]. The role of MMPs is to proteolyse dead protein debris as a natural physiological mechanism to clean the ulcer and create a favorable ground for cell growth, but unfortunately they also proteolyse and destroy cell matrix proteins. For healing to occur, young mucosal cells need to attach onto a matrix which is secreted by the mother cells before cell division so as to form a cushion for the daughter cells. This matrix is cell-specific and contains various proteins such as collagen, elastin, laminin, fibronectin, hyaluronic acid and other proteins in precise proportions. In absence of matrix, daughter cells cannot attach nor grow, and ulcers cannot heal. As MMPs destroy cellular matrix proteins, they...
hamper daughter cell attachment and block cell growth in the mucositis ulcers.

An ideal treatment should not only exert an antiseptic, antibacterial effect to clean the ulcer, but should also remove the infiltrating toxic chemicals from the ulcer to reduce the burning sensation, and neutralize all the MMPs involved in the destruction of the cellular matrix to facilitate ulcer healing.

Therefore, the aim of our research was to identify MMPs involved in Extra Cellular Matrix (ECM) destruction and to find appropriate MMP-neutralizing substances to treat oral mucositis. Since certain plant tannins and their procyanidin (PCD) fractions have strong affinity for some proteins, [4,5] we identified MMP-binding PCDs as described by Shrivastava [6]. These specific PCDs were incorporated in an osmotically active, hypertonic solution so as to create an exudation of hypotonic liquid to clean the ulcer [7]. The resulting viscous solution was filled in 20ml aluminum sprays and designated as OROSOL®.

A pilot clinical trial was conducted to evaluate the efficacy of OROSOL® against commonly used oral mucositis treatments over a period of 28 days.

2. Materials and Methods

2.1. Preliminary Research

2.1.1. Isolation of Plant Tannins (PCDs)

Initially, 44 plants or parts of plants were selected based on their tannin content to prepare extracts according to the method of Giner-Chevez et al [8]. In short, initial tannin-rich plant extract was obtained from 44 tannin-rich plants/fruits with an aqueous organic solvent containing 70% acetone and 30% water. The extracts were then successively passed through Sephadex LH-20 columns by progressively increasing the volume of methanol (60x88.5 cm), and the intended fractions were eluted to produce a dry solid. The product was identified by mass spectrometry. The extracts used for experiments contained mainly 60-80% epicatechin, catechin B1, B2, B3 and C1 fractions.

2.1.2. Research for the Proteases Involved in Chronic Wound Healing

Most of the MMPs found in abundance in chronic wounds have been identified through a slightly modified method as described by Shrivastava et al [6]. In short, OKF6/TERT-2 (TERT-2), an immortalized oral epithelial cell line (ATCC 33277), was used to model primary gingival epithelial cells. TERT-2 cells were grown in 5% CO2 at 37°C in 5% foetal calf serum (Labtech, France) in 24-well tissue culture plates (Corning, USA) for the first 24h to obtain 30-40% cell monolayer. After 24h, cell culture medium was replaced by a new serum-free medium supplemented with different concentrations of purified individual MMPs (Anaspec Inc, US). Non-cytotoxic concentrations of cell growth-inhibiting MMPs (0.5µg/ml) were then associated to obtain 95-100% cell growth inhibition (VB-MMPs). Cell growth was measured at 24, 48 and 72h using MTT vital stain, and compared with non-MMP added control cell cultures. Inhibition of cell growth was proportional to the cellular matrix destruction by MMPs.

2.1.3. Evaluation of MMP-Neutralizing Properties of Plant PCDs

To evaluate the anti-protease activity of different tannin-rich plant extracts, a fixed concentration of each PCD (50µg/ml) or an association of PCDs (25µg/ml each) was pre-incubated for 1h with VB-MMPs at concentrations of 0.5µg/ml/MMP. After 1h pre-incubation, the PCD-MMP suspension was exposed to cell cultures and cell growth was measured. Enhanced cell growth compared to non-PCD treated MMP cell cultures indicated MMP neutralization. Different PCDs were tested to select the best PCD association neutralizing 90-100% VB-MMPs. 0.66% (w/w) of these VB-PCDs (0.66%) was incorporated into an osmotically active, hypertonic solution [7] containing glycerol (84.92%) and water (14.42%) and the solution was filled into a 20ml aluminum container with an oral spray designated as Orosol®.

2.2. Clinical Evaluation

2.2.1. Aim of the Study

The aim of this study was to evaluate the safety and efficacy of Orosol® for the treatment of mucositis compared to the classical treatments commonly used in the hospitals.

2.2.2. Location

An open, multicentric, pilot study was conducted by the Bhavan Research Centre in Indore, India, between 01/2011 and 07/2011. Dr R.K. Vyas, head of the Oncology department at the Geeta Bhavan Hospital and Research Centre, was the clinical investigator.

2.2.3. Ethical Aspects

This pilot study was conducted only after the approval of Institutional Review Board/ Independent Ethical committee, agreed upon by the Indian Council of Medical research (ICMR) respecting GCP (Good Clinical Practice) and following the principles laid down in the 1964 declaration of Helsinki and later amendments. The investigative institute is authorized to conduct clinical trials and is regularly inspected by the regulatory authorities. Only the subjects who gave informed consent were included in the study. Patients not willing to try a new treatment (Orosol®) were given a classical mucositis treatment and were included in the classical treatments (CT) group.

2.2.4. Selection of Patients

The inclusion criteria were:
1. Patients willing to participate in the study,
2. Patients under recent chemotherapy, radiotherapy, or both, and suffering from mild to severe mucositis,
3. Patients accepting not to take any other mucositis treatment if allocated to the Orosol® group and to indicate the treatment they took if included in the CT group.
4. Not below 3, or above 95 years of age.

The exclusion criteria:
1. Subjects having undergone systemic antibiotic therapy in the last 2 weeks preceding recruitment, 
2. Patients in debilitating health condition.

It was decided to stop the treatment in case of any critical event.

After initial screening of 88 patients, 73 patients were selected for the study and were allocated to one of the two groups based on patient’s desire to take Orosol® or the classical treatments. 50 patients were enrolled in the Orosol® and 23 in the CT group, among which 48 and 21, respectively, completed the study as indicated in the COHORT flow chart, Figure 1.

2.2.5. Population Distribution

The distribution of a total of 69 subjects between the ages of 7 and 91 years, with mean age: 54.68 years (±20.13), is shown in Table 1.

<table>
<thead>
<tr>
<th>N°</th>
<th>Data</th>
<th>Total</th>
<th>Classical Treatment Group</th>
<th>OROSOL® SPRAY treated Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Number of Patients</td>
<td>69</td>
<td>21</td>
<td>48</td>
</tr>
<tr>
<td></td>
<td>Women</td>
<td>16</td>
<td>2</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>Squamous Cell Carcinoma</td>
<td>13</td>
<td>5</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>Salivary Adenocarcinoma</td>
<td>19</td>
<td>5</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>Laryngeal Carcinoma</td>
<td>18</td>
<td>3</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>Nasopharyngeal Carcinoma</td>
<td>8</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>2</td>
<td>Main Type of Cancer</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Stage of Cancer (T1= Initial, Tx= Terminal Stage)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Radiotherapy</td>
<td>20</td>
<td>7</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>Chemotherapy</td>
<td>17</td>
<td>3</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>Radio + Chemotherapies</td>
<td>32</td>
<td>11</td>
<td>21</td>
</tr>
<tr>
<td>4</td>
<td>Mucositis Grade at the Start</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

2.2.6. Parameters Measured

After recruitment, the medical history of the patient was recorded in the observation file. Upon evaluation of the entire baseline parameters as per the study protocol, each patient in the Orosol® group received the test product, instruction chart and symptom observation table. CT group patients followed the same protocol except for treatment product as they were authorized to take any treatment prescribed by their consultant doctor. Observations were made before treatment (T0), 20-30 minutes after 1st product application and on days 1, 2, 3, 4, 7, 14, 21 and 28 in both groups. The symptoms were scored using two different scales, as follows:

**Scale 0-4** for mucositis, presence of bacterial or fungal infections, new ulcer formation, and eating difficulty, where 0 indicates absence of symptoms and 4 severe symptoms.

**Scale 1 (minimum) to 10 (maximum)** for pain and burning sensation scores.

The mean scores of the CT group were compared with those of the Orosol® group.

The clinical research coordinator or the clinical investigator recorded the initial observations (before treatment = T0, 30min after first treatment application and on day 1) in collaboration with the patient, then the patients recorded themselves subsequent observations for each time point.

2.2.7. Treatment

Patients in the Orosol® group were asked to spray the solution over the oral mucosa 4-5 times per day during the study period. CT group patients followed treatment directions recommended by their medical advisor.

2.2.8. Statistical Methods Used for Data Analysis

Clinical data were analyzed using SAS 9.1.3. statistical program. Statistics, i.e. mean values, standard deviation (SD), minimum and maximum frequency distribution, were used for the analysis of demographic details, clinical evaluation, and medical history. If the p-value was greater than 0.05, the results were considered statistically not significant.

3. Results

Preliminarily to this clinical evaluation, we had studied the factors impeding the healing of the initial, therapy-induced mucositis ulcer, i.e. the inhibition of cellular growth by specific MMPs. We then tested our hypothesis
of specific MMPs neutralization by selected PCDs, in order to obtain a formulation for a treatment solution.

3.1. Identification of Cellular Growth-Inhibiting MMPs

*In vitro* cell culture results showed that MMPs-2, -3, -9, and -24 inhibited cell growth by 45, 52, 66, and 35%, respectively. MMP-10 reduced cell growth slightly while other MMPs had no effect on cell proliferation. When MMP-2, -3, -9 and -24 were associated with each other, 100% cell growth was blocked after 72h of culture, indicating that these four particular MMPs are involved in cellular matrix destruction in mucositis ulcers. These results show that specific MMPs are involved in the destruction of gingival epithelial cell matrix.

3.2. MMP-Neutralizing PCDs

Among 44 plant PCDs tested, only 5 PCDs effectuated 23% to 54% VB-MMP inhibition *in vitro*. All other PCDs' VB-MMP neutralization potential was below 20%. When we combined the most active PCDs with each other, maximum VB-MMP neutralization was observed, and the association of two plant extracts in particular, designated as VB-PCDs, induced 84% (±9.2%) reduction in VB-MMP activity. Although other associations of 2 or more among the selected PCDs were also active, the results were not always additive, and none of those associations proved more active than the initial VB-PCDs association, which shows that PCDs have selective affinities for MMPs. These two PCDs were therefore used to prepare Orosol®. Based on *in vitro* results, 0.66% VB-PCDs were incorporated in glycerol (84.92%) and the product’s viscosity was adjusted with water (14.42%) to be adequate for application as spray.

3.3. Population Analyses

The population distribution was homogenous between Orosol® and CT groups with respect to age, sex, and initial oral mucositis symptoms. The number of patients was higher in the Orosol® group (n=48) than in the CT group (n=21) as allocation, before study outset, was dependent on the patient’s choice of treatment. Moreover the efficacy of other, classical, treatments is fairly well known while there is no topical MMP-inhibitor to compare the efficacy of Orosol® to. For ethical reasons, it was not possible to include an untreated (placebo) control group.

Above 80% patients (58/69) were being treated either for squamous cell carcinoma, salivary adenocarcinoma, laryngeal cancer or nasopharyngeal cancer. Another 11 patients had either: breast cancer (1 in CT group and 3 in Orosol® group), plasctostoma (2 in each group), skin cancer (1 in each group), or other type of cancer (1 in the Orosol® group). A majority of patients (>65%) were between T2 and T4 stage of cancer and were receiving either radiotherapy or chemotherapy. The distribution of pathology and cancer treatment was fairly identical in both groups. Among 21 patients in the CT group, 6 were receiving only chlorhexidine mouthwashes, 8 were under PTA (polymyxin, tobramycin, amphotericin) antibiotic therapy, 4 were treated with local anaesthetics (lingocaine), while 3 were treated with strong opioid analgesics.

3.4. Effect on Oral Mucositis Parameters

Results are shown in Table 2.

All the results for Orosol® group show a statistically significant difference compared to CT group (p<0.05) between days 1 and 28, for all the parameters.

3.5. Oral Mucositis Grade

The initial mean score of mucositis was slightly higher in the Orosol® group (2.33) compared to the CT group (2.67) but this difference was not significant on day 1. As Figure 2 shows, a progressive reduction in mucositis score was noticed in the Orosol® group between days 2 and 4, as on day 4 the mean score was only 1.58 in the Orosol® group compared to 2.62 in the CT group. Orosol® group patients showed a gradual reduction in the mean mucositis score up to day 28 (mean score 0.83, indicating nearly 70% reduction compared to pre-treatment), with nearly 60% lesser severity in the Orosol® group on day 28 compared to CT group (mean score 2.10 on day 28). Mucositis score was only slightly reduced in the CT group by the end of the study (-10% compared to pre-treatment). Results clearly indicate that Orosol® significantly diminishes the severity of oral mucositis with progressive reduction throughout the study, although total recovery was not observed over the study period.

![Effect on Overall Mucositis Grade : 0-4 scale](image)

**Figure 2.** Evolution in Overall Mucositis Grade in CT and Orosol® groups: mean values and standard deviations

3.6. Pain

Treatment effects on pain can be observed in Figure 3. The mean pain intensity score was very high (7.1/10) in both groups before start of treatment, but right after the 1st application, a remarkable pain reduction was observed in the Orosol® group (mean score 4.71 compared to 7.71 in the CT group), indicating a 34% reduction in severity as early as 20-30 minutes after 1st product application. In the CT group, the pain intensity started decreasing only after day 3 (mean score 6.91/10 on day 3) with a very slight and progressive reduction up to day 28 (mean score 5.71/10 on day 28). Patients noted that local anaesthetics were the most efficient to suppress the pain instantly, but the effect was observed only for 30 minutes following each product application. In the Orosol® group, the pain intensity continued decreasing rapidly up to day 3 (mean score 3.08/10 on day 3 indicating a 55% lesser severity than in the CT group).
but thereafter pain reduction was very slight and progressive up to day 28, with a 70% global reduction in severity compared to pre-treatment. These results show that Orosol® is effective and instantaneous in reducing mucositis pain during the first 3-4 days of treatment but the product does not suppress the pain totally.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Before Tr = 10</th>
<th>20-30 min</th>
<th>Day 1</th>
<th>Day 2</th>
<th>Day 3</th>
<th>Day 4</th>
<th>Day 7</th>
<th>Day 14</th>
<th>Day 21</th>
<th>Day 28</th>
</tr>
</thead>
<tbody>
<tr>
<td>CT</td>
<td>2.33 (±0.02)</td>
<td>2.33 (±0.02)</td>
<td>2.19 (±0.02)</td>
<td>2.19 (±0.02)</td>
<td>2.33 (±0.02)</td>
<td>2.62 (±0.02)</td>
<td>2.52 (±0.02)</td>
<td>2.24 (±0.02)</td>
<td>2.14 (±0.02)</td>
<td>2.10 (±0.02)</td>
</tr>
<tr>
<td>% Change/T0</td>
<td>0%</td>
<td>-6%</td>
<td>0%</td>
<td>+3%</td>
<td>-3%</td>
<td>-11%</td>
<td>-17%</td>
<td>-13%</td>
<td>-19%</td>
<td>-20%</td>
</tr>
<tr>
<td>Orosol®</td>
<td>2.67 (±0.91)</td>
<td>2.46 (±0.85)</td>
<td>2.30 (±0.99)</td>
<td>2.05 (±0.00)</td>
<td>1.73 (±0.86)</td>
<td>1.58 (±0.78)</td>
<td>1.41 (±0.73)</td>
<td>1.10 (±0.66)</td>
<td>0.93 (±0.52)</td>
<td>0.83 (±0.52)</td>
</tr>
<tr>
<td>% change/T0</td>
<td>-8%</td>
<td>-14%</td>
<td>-23%</td>
<td>-35%</td>
<td>-41%</td>
<td>-47%</td>
<td>-59%</td>
<td>-64%</td>
<td>-69%</td>
<td>-69%</td>
</tr>
<tr>
<td>% Diff O/C</td>
<td>+14%</td>
<td>+5%</td>
<td>+5%</td>
<td>+11%</td>
<td>+20%</td>
<td>+8%</td>
<td>-12%</td>
<td>+15%</td>
<td>-36%</td>
<td>-37%</td>
</tr>
</tbody>
</table>

Table 2. Mean Scores Recorded for each Parameter in the Classical Treatments (CT) or Orosol® (O) Group (± Standard Deviation of the Mean), % of Change Observed in each Group Compared to T0, and % Difference in Severity Scores in Orosol® Group (O) Compared to the Classical Treatments Group (CT).

3.7. Burning Sensation

Oral burning sensation was slightly higher (+6%) in the Orosol® group compared to the CT group before treatment but was markedly diminished in the Orosol® group 20-30 minutes after 1st product application. Treatment effects are seen in Figure 4: the mean burning sensation score was reduced by 41, 47, 58 and 66% on days 1, 2, 3, and 4, respectively, but reduction rate lessened slightly after day 7, to remain around a constant 70-75%, from then on. All patients noticed a slight initial burning sensation for about 1-2 minutes after each Orosol® application, followed by a temporary sensation.

![Figure 3. Evolution in Pain Sensation in CT and Orosol® groups: mean values and standard deviations](image-url)
of salivation, and a reduction in pain which remained constant between two product applications.

**Figure 4.** Evolution in Burning Sensation in CT and Orosol® groups: mean values and standard deviations

### 3.8. Infection

The mean infection (bacterial and fungal) score was lower in the Orosol® compared to the CT group (-13%) at study outset. As shown in **Figure 5**, the infection score remained rather constant in the CT group but showed a progressive reduction in the Orosol® group after 1st application (-20% compared to pre-treatment) up to day 21 (-77% compared to pre-treatment, with a 79% lower severity compared to the CT group). Results indicate however that some infection always persists even after 3 weeks of Orosol® treatment.

**Figure 5.** Evolution in Infection rates in CT and Orosol® groups: mean values and standard deviations

### 3.9. New Ulcer Formation

Although some ulcers healed, new ulcers also appeared during the study, and the number of ulcers remained nearly constant in the CT group throughout the study, as seen in **Figure 6**. Investigators recorded a reduction in the size of the ulcers in the Orosol® group and a slight (between 20% and 30% from day 4 onwards, compared to pre-treatment values) but statistically significant ($p<0.05$) reduction in the appearance of new ulcers, with a matching 20-30% lesser severity compared to the CT group, from day 7 onwards.

**Figure 6.** Evolution in New Ulcer Formation in CT and Orosol® groups: mean values and standard deviations

### 3.10. Nutrition

At the start of the study, most patients were on a soft or liquid diet (mean score between 2.6 to 2.8 on a 0-4 scale). Evolution in food intake ability is showed in **Figure 7**. The score was not considerably changed in the CT group during the study, with only a 9% reduction on day 28 compared to before treatment mean value. In the Orosol® group, the overall reduction in mucositis led to a slow and progressive improvement in patient’s capacity to eat a soft diet as the initial score of 2.81 progressively decreased to 1.90 on day 4, and 1.50 on day 28, indicating a 47% global reduction in eating impairment, compared to initial values. As a result, 85% patients in the Orosol® group were able to take in a soft diet at the end of the study.

**Figure 7.** Evolution in Eating Difficulty in CT and Orosol® groups: mean values and standard deviations. Scores represent: 1= solid diet; 2= soft diet; 3= liquid diet; 4= no food intake

### 3.11. Safety

Except for the initial burning sensation followed by excessive salivation during the first 1-2 minutes after each Orosol® application, no other undesirable events were attributed to the treatments in any of the patients. This initial burning sensation may be related to the instant exudation of hypotonic liquid following each Orosol® application.

These results clearly indicate that Orosol® is highly effective in reducing pain, burning sensation, infection and overall oral ulcerative condition, and therefore to treat mucositis. Although the product is less effective in inhibiting the formation of new ulcers, it can be considered as a new therapeutic approach to treat oral mucositis of multiple origins.

### 4. Discussion

Oral mucositis is one of the most debilitating side effects of chemotherapy and radiotherapy, particularly for
those patients undergoing treatment for head and neck cancers. It seriously affects the patient’s well being, the total cost of treatment, and the chances of success of the anticancer treatment [9]. Despite its being regularly reported and considered as one of the serious causes of the failure of cancer treatments, relatively little efforts are made to understand the complexity of mucositis injury and to search an effective drug [10]. Numbers of symptomatic treatments are being proposed, but none of these is considered really effective. All the anticancer treatments are directed to inhibit the growth of cancer cells, but currently there is no treatment which can selectively stop the multiplication of cancer cells without affecting the growth of normal healthy cells. As oral mucosa is one of the fastest growing organs in our body, the oral mucosa cells are extremely sensitive to any anti-mitotic drug, and the absence of cell growth initially manifests itself as a small ulcer on the mucosa surface. Our body has a normal tendency to repair any injury, but the presence of anti-mitotic drugs hampers cell growth, so that the injury increases in size, eventually gets infected by bacteria, fungus and other oral microorganisms, and forms a severe ulcer. Damaged blood vessels around the ulcer surface liberate circulating cancer treatment drugs into the ulcer, causing the severe sensation of pain and burning. Bacterial endotoxins aggravate this sensation and condition, and patients suffer from severe mucositis. Due to the presence of cytostatic or cytotoxic drugs in the body, or to the radiotherapy treatment, normal cell growth and wound repair process are blocked, and ulcers become chronic.

Cell and tissue destruction produces protein debris, which requires cleaning to prepare a favorable environment for cell growth. This is the reason why different inflammatory cells in the ulcer produce various MMPs, proteolytic enzymes to break down the protein debris into smaller molecules in order to clean the injury. More than 25 MMPs have already been identified in chronic ulcers with a concentration 10-30 fold higher than what is observed in an acute wound [11]. Unfortunately these MMPs cannot distinguish between an unwanted and a useful protein and thus destroy the cellular matrix proteins, essential for cell growth and ulcer healing [12].

Oral mucositis ulcer healing requires cell growth, in order for which cells need to attach onto a matrix. This matrix is a complex association of different proteins such as collagen, elastin, fibronectin, laminin, hylauronic acid, and proteoglycans, which are all protein molecules. More and more recent evidence suggests that MMPs, such as collagenase and elastase, destroy cellular matrix proteins in chronic wounds [13]. Some recent studies are seriously considering the involvement of MMPs in the development of oral and intestinal mucositis ulcers, [14,15] but until recently there have been no studies evaluating the levels of key MMPs in mucositis ulcers. A few in vitro and animal studies have shown that levels of MMP-2, -3, -9, and -12 increase dramatically during mucositis, [15,16] so that natural tissue inhibitors of MMPs (TIMPs) may play an important role in the treatment of mucositis [17]. Current knowledge also points out that different cellular matrix-degrading MMPs are involved depending on the type of wound, i.e. acute versus chronic [3]. Since the type and concentration of MMPs vary according to the stage, type and location of the injury, and as multiple MMPs are present, it is nearly impossible to block all those MMPs simultaneously with one single chemical drug. [18] Due to the affinity of certain polymers and plant PCDs for proteins, and their capacity to bind with and neutralize MMPs, [19,20] these substances have already been suggested for the treatment of chronic ulcers [17,21] and periodontitis, [22,23] and to block certain specific protein receptors on the cell surface [24]. But taking into consideration the concentration and number of MMPs found on the surface of a chronic ulcer, it was important to identify the precise MMPs involved in mucositis ulcer matrix degradation and to select an association of protein-binding tannins or procyanidins (PCDs) capable of blocking all the oral mucosa matrix-destroying MMPs simultaneously.

5. Conclusions

We studied cell growth inhibition by cellular matrix-degrading oral mucositis MMPs and identified cellular matrix-degrading oral mucositis MMPs and the corresponding PCDs that are capable of neutralizing them. Orosol® contains those specific MMP-inhibitors in an osmotically active hypertonic solution which creates an exudation of hypotonic liquid from the inner part of the ulcers so as to replace the normal physiological functions of MMPs. This instant mechanical effect of Orosol® helps to eliminate not only the dead protein debris and microbial contaminants but also the toxic chemicals present in the mucositis ulcer. At the same time, PCDs bind with and neutralize MMPs, creating a favorable ground for cell growth and ulcer healing. Although this novel approach in treating oral mucositis ulcers is extremely efficient to reduce pain, burning sensation, infection, and size of the ulcers, it has no effect on the development of new ulcers as long as the oral mucosa cells are exposed to cytostatic or anti-mitotic therapy.

The results of this pilot clinical trial prove that specific MMP inhibition is the key approach to treat oral mucositis and requires further exploration to propose an efficient treatment for millions of patients suffering from oral mucositis.

Funding Acknowledgment

This research was entirely supported by VITROBIO Research Institute, ZAC de Lavaur, 63500 Issoire, France, without any sponsor.

Personal Acknowledgements

We thank Dr R.K.Vyas, Head of Oncology department, Geeta Bhavan Hospital & Research Center, Indore, India, for his participation in this study as clinical investigator and coordinator.

List of Abbreviations

CT: Classical Treatments
References


