**Outcome of Local Application of Amifostine (WR-1065) on Epirubicin-induced Oral Mucositis. A Phase II Study**


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**Abstract.** Background: Intravenous administration of amifostine reduces chemotherapy-induced toxicity. Preclinical experiments showed a reduction in radiation-induced mucositis after local application of the active metabolite of amifostine (WR-1065). This study evaluated the effect of local application of WR-1065 on chemotherapy-induced oral mucositis. Patients and Methods: Non-small cell lung cancer patients treated with gemcitabine and epirubicin every 3 weeks for a maximum of five cycles were included. WR-1065 was administered during the second and third cycle as an oral rinse. Oral mucositis evaluation included WHO toxicity grading, a validated oral mucositis assessment scale (OMAS) and a questionnaire. Results: Twenty-four patients were evaluated for at least one control and one rinse cycle. Mucositis scores, pain and feeding difficulties increased from day 1 to day 15, and were not significantly different between the control and rinse cycles. Local application of WR-1065 leads to detectable quantities of WR-1065 in epithelial mucosa cells. A negative correlation between the WR-1065 concentration and OMAS score was found. Conclusion: No clinical detectable influence of WR-1065 on oral mucositis was found.

Oral mucositis is an inflammatory-like change of the oral mucosa due to cytotoxic chemotherapy or radiotherapy. The type of antineoplastic treatment and patient-related factors influence the incidence and severity of oral mucositis. The onset is usually between 3 and 7 days after chemotherapy and the duration is highly variable. Especially in patients receiving high-dose chemotherapy followed by bone marrow or peripheral stem cell transplantation, mucositis can be dose-limiting (1). The pathogenesis of oral mucositis is not fully understood, but is thought to involve direct and indirect mechanisms. The direct toxic effect of cytostatic agents on rapidly dividing cells of oral epithelium can result in mucosal atrophy, erythema and ulceration. Indirect stomatotoxic effects are caused by release of inflammatory mediators, loss of protective salivary constituents and therapy-induced neutropenia. Bacteria, fungi and viruses can superimpose secondary infections on the damaged mucosa. Mucositis is proposed to develop in four consecutive phases: i) the inflammatory/vascular phase (release of free radicals and cytokines); ii) the epithelial phase (reduced epithelial renewal) with atrophy and ulceration; iii) the ulcerative/bacterial phase (colonisation mixed flora, causing release of endotoxins) with further tissue damage by stimulation of cytokines; iv) the healing phase (2).

Mucositis causes major discomfort in patients. Pain and restriction of normal feeding and drug intake are the most important discomforts. In severe stages of mucositis secondary infection of mucosal ulcers can provide a port of entry for micro-organisms into the circulation, which can lead to life-threatening sepsicaemia, especially in myelosuppressed patients. It is worthwhile to investigate strategies to prevent oral mucositis since the actual regimens for mucositis prevention are mainly palliative. Local and systemic analgesics are applied for pain relief, while antimicrobial agents are applied for bacterial or fungal infections or for prevention.

Cytoprotective agents, such as amifostine (Ethylol®, WR-2721), can reduce the toxicity induced by radiotherapy or chemotherapy. Amifostine is a pro-drug, which is active as a protective agent when dephosphorylated by alkaline phosphatase to its active metabolite WR-1065. WR-1065 is preferentially taken up into normal rather than neoplastic cells because of the higher alkaline phosphatase activity, better vascularisation and higher pH of normal tissue. Once inside the cell, WR-1065 protects against chemotherapy- and radiation-induced damage by scavenging free radicals.

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donating hydrogen ions to free radicals, depleting oxygen, and direct binding and inactivation of cytotoxic drugs (3,4). Intravenous administration of amifostine provides protection against a broad range of cytotoxic agents. Reduction in haematological or non-haematological toxicity is described for cisplatin, carboplatin, doxorubicin, paclitaxel and 5-fluorouracil (5-8). Prevention of mucositis is mainly described in head and neck cancer patients treated with radiotherapy (9-11).

Pre-clinical experiments showed a reduction in radiation-induced mucositis after local application of amifostine. Topical application of 50 mg amifostine to the buccal mucosa in mice reduced the severity of radiation-induced oral mucositis, without any toxicity (12). However, no prevention against radiation-induced proctitis and colitis was found after rectal administration of amifostine doses between 100 and 450 mg/enema. (13). Reasons for failure to protect the rectosigmoid mucosa may be related to the method of administration (the rectum was not empty), and the relatively long period (45 minutes) between application of amifostine and the onset of radiation.

A pilot study in patients with stage IIIb or IV non-small cell lung cancer (NSCLC) evaluated the feasibility of 125 mg WR-1065 as a mouthwash. Before administration, 200 mg WR-2721 was ex vivo converted to WR-1065. No systemic side-effects were observed and WR-1065 was well-tolerated. In this study, WR-1065 was detectable in washed, isolated, vital mouth mucosa cells at a concentration of 3.7-19.9 ng/10^6 cells (14). Previous in vitro experiments showed that a cellular concentration of 13 ng WR-1065 in 10^5 mouth mucosa cells induced radioprotection (15,16). Taken together, these findings suggest that 200 mg WR-2721 (10 ml 0.09 M) or 125 mg WR-1065 (10 ml 0.09 M) can be safely administered, and the reached cytoprotective concentration achieved might be effective in the treatment of oral mucositis.

The aim of this phase II trial was to evaluate the effect of local application of WR-1065, the active compound of amifostine, on oral mucositis in NSCLC patients treated with epirubicin and gemcitabine. The effect of WR-1065 on mucositis was the primary end point of this study. The secondary end point was to determine WR-1065 in oral epithelial mucosa cells.

Patients and Methods

Patient selection. Patients (≥18 years) with a histologically or cytologically proven diagnosis of unresectable stage III or IV NSCLC were eligible for this study. Exclusion criteria were: child-bearing potential without effective means of contraception; pregnancy or lactation; presence of other malignancies; presence of ulcerative lesions in the oral cavity or any grade of mucositis in the last 4 days; Sjögren’s syndrome; infection requiring systemic antibiotics within the previous 14 days; significant renal dysfunction (serum creatinine levels > 1.5 times the normal acceptable range (62-10^9 umol/L) or creatinine clearance less than 50 mL/minute); haematological disorders (leukocytes < 3.0x10^9/L, neutrophils < 1.5x10^9/L, Hb < 5.0 mmol/L); use of topical oral disinfectants within the previous two weeks; or use of other investigational drugs within the previous 30 days. The medical ethics review committee approved the protocol and all eligible patients gave written informed consent before study entry.

Treatment. Gemcitabine (1125 mg/m^2) was administered in 250 mL of 0.9% NaCl by a 30-minute infusion on days 1 and 8 of each 21-day cycle. Epirubicin (100 mg/m^2), in 50 mL of 0.9% NaCl was given as an intravenous bolus injection within 5 minutes on day 1 (after gemcitabine administration). Treatment consisted of a maximum of five cycles and was stopped earlier in case of tumour progression, intolerable toxicity or patient's request. Ondansetron 8 mg and dexamethasone 8 mg were used as anti-emetics twice a day on days 1, 2 and 8. During the second and third cycle, WR-1065 (10 ml, 20 mg/mL) was administered as an oral rinse. Before administration, amifostine (WR-2721) was converted to WR-1065 ex vivo (WR-2721 at 37°C for 1 hour, pH 3.5), because alkaline phosphatase concentrations in the mouth are low. The oral rinse was used 15 minutes before and 5 minutes after epirubicin infusion. Patients were instructed to rinse and gargle for 1 minute and subsequently to spit out.

Treatment evaluation. During the study period, evaluation was conducted on days 1, 8 and 15 of each cycle by a dental hygienist or a physician. Oral mucositis was evaluated using World Health Organization (WHO) toxicity grading and the Oral Mucositis Assessment Scale (OMAS) (17). OMAS evaluates nine regions of the oral cavity for erythema and the presence and size of pseudomembranes or ulcerations. The value of OMAS at any given assessment is obtained by summing the erythema and ulceration/pseudomembrane subscores at each site and then averaging these scores across all nine sites. The OMAS score ranges from 0-5.

On days 1, 8 and 15 of each cycle patients filled in a questionnaire. This questionnaire consisted of 100 mm visual analogue scales for oral pain (ranging from ‘no pain, 0 mm’ to ‘very severe pain, 100 mm’) and swallowing difficulty (ranging from ‘no impact on swallowing, 0 mm’ to ‘extreme impact on swallowing, 100 mm’) and one multiple-choice question about eating function (with four levels of functioning: ‘normal’, ‘soft foods only’, ‘liquids only’, or ‘no oral intake possible’).

For determination of the cell viability of oral mucosus cells, an oral washing was performed before administration of WR-1065 and cytostatics, on days 1, 8 and 15 of each cycle (18,19). To obtain an oral washing, patients gargled and rinsed their mouth for 30 seconds with 10 mL sterilized 0.9% NaCl solution and then spat it out into a tube. This expectorate was centrifuged within 10 minutes of collection (190 g, 10 min, 4°C) and the supernatant was removed. The cell suspension was washed with 10 mL of 0.9% NaCl and centrifuged again to eliminate salivary fibres. Cell pellets were resuspended in 1 mL RPMI (Gibco, Paisley, UK) containing 5% fetal calf serum. Subsequently, 50 µL suspension and 50 µL trypan blue dye (0.4% in 0.9% NaCl) were mixed and cell counts were performed to calculate the percentage of viable cells.

For determination of WR-1065, an oral washing was obtained before and 15 minutes after treatment with WR-1065 during the
second and third cycle. Subsequently, the expectorate was subdivided into two portions. For determination of cell viability 1 mL was used. The remaining 9 mL was centrifuged and washed according to the above-mentioned procedure. Thereafter, the cell pellet was stored at -80°C. WR-1065 concentrations were determined by high performance liquid chromatography as described by Korst (20).

Statistical analysis. Based on the results of a phase II study with epirubicin and gemcitabine every 3 weeks, WHO grade 2-3 mucositis is expected to occur in the first and/or fourth cycle (control cycles) in 35% of patients (21). The incidence in the second and/or third cycle (rinse cycles) is expected to be 10%. Based on the McNemar test on discordance, 20 patients are needed to detect a 25% difference with a power of 85% and a two-sided significance level of 5%. With a drop-out of about 15%, 25 patients have to be included.

Data are analysed using a linear mixed effects model. Respondent and cycle number are considered to be nested factors, days are modelled linear within cycles. When a response variable had a skewed distribution, the data was log-transformed. Summary measures were made and compared for cycles with and without mouthwash. To exclude the impact of the order of the control and rinse cycle in relation to the response variables, a crossover design was used for analysis. Therefore, twelve patients with a control cycle followed by a rinse cycle, and twelve patients with a rinse cycle followed by a control cycle were selected. Spearman’s correlation coefficient was calculated for correlation of the WR-1065 concentration and mucositis.

Results

Patient characteristics and treatment. From December 2000 till April 2002, 29 patients were included. Due to discontinuation of chemotherapy, five patients dropped out earlier. The remaining 24 patients had at least two evaluable chemotherapy cycles, one with WR-1065 rinse and one without. The patients evaluated in this study comprised 20 men and 4 women, with a mean age of 61 years (range 46-73). Because not all patients had four evaluable cycles, the first chemotherapy cycle was used for all patients as a control cycle. The cycle with the fewest missing data was used as WR-1065 rinse cycle; 19 times cycle two, 4 times cycle three and 1 time cycle five.

Mucositis. During the first cycle, 21% of the patients experienced mucositis WHO grade 2. The mean mucositis score increased from day 1 to day 15 in both the control and rinse cycle (Table I). During the total treatment period, none of the patients experienced WHO mucositis score grade 3 and 4. No significant differences in WHO and OMAS mucositis score were found between control and rinse cycles.

Pain and feeding. A significant difference for pain was found between the consecutive days within the same cycle, with increasing pain in time (p<0.01). No significant difference for pain was found between the control and rinse cycle (Table I). For difficulties in swallowing and eating function, no significant differences were found between the control and rinse cycle (Table I).
Table III. Percentage viable epithelial cells (sd).

<table>
<thead>
<tr>
<th></th>
<th>Day 1 before rinsing</th>
<th>Day 1 after rinsing</th>
<th>Day 8</th>
<th>Day 15</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>56 (18.0)</td>
<td>-</td>
<td>59 (18.8)</td>
<td>56 (10)</td>
</tr>
<tr>
<td>Rinse</td>
<td>56 (14.1)</td>
<td>53.6 (20.0)</td>
<td>62 (12.7)</td>
<td>58 (11.2)</td>
</tr>
</tbody>
</table>

The results of the crossover design are shown in Table II AB. These data suggest a possible influence of the sequence and cycle number of the control and rinse cycle. However, analysis of the data summarised in Table II with use of a linear mixed effects model shows no significant differences between the control and rinse cycle for all response variables (OMAS and WHO mucositis score, pain, swallowing and feeding).

Viability of mucosal epithelial cells. The mean percentage viable epithelial cells at the start of the control cycles (56 ± 18%) was comparable with this percentage at the start of the rinse cycles (56 ± 14%). No significant differences were found between the consecutive days within the same cycle. Nor was a significant difference found in the viability of mucosal epithelial cells between the control and rinse cycle (Table III).

Determination of presence of WR-1065. It was possible to determine the presence of WR-1065 in washed, isolated, vital mouth mucosa cells in 22 out of 24 patients. The cell pellet was lost in one patient; another patient had an interfering analytical peak, which made determination of WR-1065 impossible. The median cellular concentration of WR-1065 was 11.9 ng/10^5 viable cells (range 0.09-3821.7 ng/10^5). Eleven patients had a mean cellular concentration < 13 ng/10^5 viable cells. A significant negative correlation was found between the concentration of WR-1065 and the OMAS mucositis score (r=-0.54, p=0.012).

Discussion

This study shows no clinical effect of local application with WR-1065 on oral mucositis in patients with unresectable stage III or IV NSCLC. Nevertheless a significant negative correlation was found between the WR-1065 concentration in mouth mucosa cells and the OMAS mucositis score, meaning that a higher cellular concentration of WR-1065 leads to less mucositis.

The cellular concentration of WR-1065 found in mouth mucosa cells after the first mouthwash with 125 mg WR-1065 had a large range (0.09 to 3821.7 ng/10^5). In vitro studies showed that after a five-minute incubation period with 4 mmol/l WR-1065, a concentration of 13 ng/10^5 cells was detectable and effective (16). By adding 4 mmol/l amifostine in combination with alkaline phosphatase to V79-171 cells, 40% of the cells were protected from damage caused by radiotherapy (8 Gy) (15). In the current study, a cellular concentration of WR-1065 < 13 ng/10^5 (range 0.09-7.1) was found in 11 patients. This concentration was lower than the concentration found by Calabro-Jones et al., which was necessary for a protective effect.

Another reason for finding no clinical effect on mucositis and the low cellular concentrations might be the uptake of WR-1065. Following intravenous administration, amifostine is rapidly cleared from the plasma (22). The rapid clearance of amifostine is largely due to the fast conversion of amifostine to its active metabolite, WR-1065. An animal study showed that maximum tissue concentrations of WR-1065 occurred within 5 to 15 minutes after amifostine injection (23). Based on these results, amifostine should be applied a short period (15 to 30 minutes) before chemotherapy. Therefore, WR-1065 was given 15 minutes before epirubicin infusion in this study. The uptake of WR-1065 is dependent on the temperature, the pH and contact time (16). The inter-individual differences in cellular concentrations of WR-1065 might be caused by differences in oral temperature and pH. Although patients were instructed not to eat or drink during the epirubicin infusion, a physiological difference in the pH of saliva could result in different WR-1065 concentrations.

The low incidence of the experienced mucositis might be another reason for finding no clinical effect. Only 21% of the patients experienced WHO grade 2 mucositis during the first cycle, though at least 35% of grade 2 or more was expected (21). During the total treatment period, none of the patients experienced a WHO mucositis grade 3 and 4. While in the study of Wachters et al. 12% of the patients experienced WHO mucositis grade 3 and 2% grade 4 (24). The low cellular concentration of WR-1065, together with the relatively low severity of mucositis, may be the reason for not finding a significant clinical effect on the presence of mucositis.

The results of this study indicate that, for prevention of oral mucositis, a higher cellular concentration of WR-1065 is necessary. Therefore, future studies should aim on a higher cellular concentration of WR-1065. This could probably be realised by a higher rinsing frequency, extended rinsing time and/or increasing the concentration of WR-1065. Another aspect is the evaluation of the pH in patients. It might be interesting to better characterise the influence of the pH value on the uptake of WR-1065 into mouth mucosa cells.

In conclusion, local application of WR-1065 is feasible and leads to detectable quantities of WR-1065 in washed, isolated, vital mouth mucosa cells. An effective cellular concentration of WR-1065 was found in only 50% of the patients. A negative correlation was found between the concentration of WR-1065 and the OMAS mucositis score.
No clinical detectable difference in mucositis between the control and rinse cycle was found. The low cellular concentration of WR-1065 and the low incidence and severity of mucositis might be the reason for finding no clinical effect of WR-1065.

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References


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