5-Fluorouracil sensitivity varies among oral microorganisms

Eline Vanlancker, Barbara Vanhoecke, Rozel Smet, Ruben Props and Tom Van de Wiele

Center for Microbial Ecology and Technology (CMET), Ghent University, Coupure Links 653, 9000 Ghent, Belgium

5-Fluorouracil (5-FU), a commonly used chemotherapeutic agent, often causes oral mucositis, an inflammation and ulceration of the oral mucosa. Micro-organisms in the oral cavity are thought to play an important role in the aggravation and severity of mucositis, but the mechanisms behind this remain unclear. Although 5-FU has been shown to elicit antibacterial effects at high concentrations (>100 µM), its antibacterial effect at physiologically relevant concentrations in the oral cavity is unknown. This study reports the effect of different concentrations of 5-FU (range 0.1–50 µM) on the growth and viability of bacterial monocultures that are present in the oral cavity and the possible role in the activity of dihydropyrimidine dehydrogenase (DPD), an enzyme involved in 5-FU resistance. Our data showed a differential sensitivity among the tested oral species towards physiological concentrations of 5-FU. Klebsiella oxytoca, Streptococcus salivarius, Streptococcus mitis, Streptococcus oralis, Pseudomonas aeruginosa and Lactobacillus salivarius appeared to be highly resistant to all tested concentrations. In contrast, Lactobacillus oris, Lactobacillus plantarum, Streptococcus pyogenes, Fusobacterium nucleatum and Neisseria mucosa showed a significant reduction in growth and viability starting from very low concentrations (0.2–3.1 µM). We can also provide evidence that DPD is not involved in the 5-FU resistance of the selected species. The observed variability in response to physiological 5-FU concentrations may explain why certain microbiota lead to a community dysbiosis and/or an overgrowth of certain resistant micro-organisms in the oral cavity following cancer treatment.

INTRODUCTION

5-Fluorouracil (5-FU) is a commonly used chemotherapeutic agent for colon and breast cancers. It is an antimetabolite that inhibits human thymidylate synthase (TS) and is incorporated in DNA and RNA (Grem, 2000; Longley et al., 2003). One of the main side effects of 5-FU treatment is oral mucositis, an inflammation and ulceration of the mucosa of the oral cavity (Peterson & Sonis 1982; Carnel et al., 1990). It has a major impact on affects the quality of life of patients, including problems with eating, speaking and drinking, and treatment mainly is concentrated on pain relief and oral hygiene (Villa & Sonis 2015).

Along the gastrointestinal tract, micro-organisms play an important role in sustaining homeostasis and health (Aziz et al., 2013). Evidence is also emerging that micro-organisms are involved in the development and severity of 5-FU-induced mucositis (Stringer & Logan, 2015; Vanhoecke et al., 2015). Changes in the composition of the oral micro-organisms have been reported for chemotherapy treatment in human studies (Lucas et al., 1997; Napeñas et al., 2010) and for 5-FU specifically in a rat study (Von Bültzingslöwen et al., 2003), with a shift towards more Gram-negative rods and bacterial translocation to cervical and mesenteric lymph nodes following treatment with 5-FU. A number of studies have been looking at the antibacterial effect of 5-FU on monocultures of mainly pathogenic strains such as Pseudomonas, Klebsiella and Staphylococcus (Wright & Matsen, 1980; Ueda et al., 1983; Bodet et al., 1985; Takahata et al., 1986). Although 5-FU was shown to have substantial antibacterial effects, the tested concentrations in these studies were much higher than what is usually present in vivo. Notwithstanding the fact that after an intravenous bolus injection with 5-FU, plasma concentrations in cancer patients can reach some hundred micromolars, the plasma levels eventually drop to 15–30 µM after 30 min and to 0 µM after 2 h (Casale et al., 2004; Kosovec et al., 2008),

Abbreviations: 5-FU, 5-fluorouracil; DPD, dihydropyrimidine dehydrogenase; µ, growth rate; OD, maximal optical density; TS, thymidylate synthase.

Two supplementary figures and tables are available with the online Supplementary Material.
owing to the short half-life time (6–22 min) of 5-FU (Bocci et al., 2000). In the case of continuous infusion with 5-FU, the plasma concentrations are much lower, ranging from 3 to 10 µM and kept for a longer time period (24 h) (Jouila et al., 1999; Takimoto et al., 1999). It is, therefore, of interest to investigate the putative antimicrobial effect of physiologically relevant 5-FU concentrations on commensal micro-organisms in the context of oral mucositis.

One of the main reasons for the short half-life time of 5-FU is the presence of dihydropyrimidine dehydrogenase (DPD) in humans which breaks down 80% of the 5-FU to dihydrofluorouracil in the liver (Diasio & Harris, 1989). Patients with decreased DPD activity are more sensitive to 5-FU and are more likely to develop side effects, like mucositis, neutrotoxicity, and myelosuppression (Diasio et al., 1988; Harris et al., 1991; Takimoto et al., 1996). Interestingly, some microbial species also possess DPD activity (Hidese et al., 1996). This might play a role in their sensitivity to 5-FU.

For this study, several representative species of the oral cavity were selected. Streptococcus salivarius, Streptococcus oralis and Streptococcus mitis were included, since Streptococcus is the most dominant genus present in the oral cavity (Dewhirst et al., 2010). Also, less abundant Lactobacillus species, Lactobacillus salivarius, Lactobacillus oris and Lactobacillus plantarum, were included because of their ability to produce lactic acid (Marsh & Martin, 1999). Fusobacterium nucleatum was included, as it is important in biofilm formation in the oral cavity (Zijlje et al., 2010). Furthermore, the non-pathogenic Neisseria mucosa was included, as it can cause bacteraemia in neutropenic patients developing oral mucositis (Mechergui et al., 2014). As pathogens can play an important role in mucositis, we also included Streptococcus pyogenes, Pseudomonas aeruginosa and Klebsiella oxytoca. S. pyogenes is a common oral pathogen (Wescombe et al., 2012), whereas P. aeruginosa and Klebsiella were recorded in the oral cavity of chemotherapy-treated patients (Panghal et al., 2012). Furthermore, oral mucositis has been associated with an increase in Klebsiella (Marsh & Martin, 1999). In this study, we studied the variation in sensitivity of different relevant oral species towards various 5-FU concentrations in the context of oral mucositis. Also, the putative role of DPD in microbial resistance to 5-FU was evaluated.

**METHODS**

**Chemicals.** A filter-sterilized stock solution of 100 mM 5-FU (Sigma Aldrich) was prepared in DMSO and further diluted to 50, 25, 12.5, 6.3, 3.1, 1.6, 0.8, 0.4, 0.2 and 0.1 mM. A filter-sterilized stock solution of 250 mM uracil (Sigma Aldrich) was prepared in DMSO and further diluted to 125, 100, 50, 32, 16, 12.8 and 6.4 mM. Stock solutions were further diluted (1:1000) in culture medium for the experiments.

**Micro-organisms and culture conditions.** All monocultures were obtained from the Belgian Co-ordinated Collection of Micro-organisms/ LMG bacteria collection. *S. oralis* (LMG 14553), *S. salivarius* (LMG 11489), *S. mitis* (LMG 14557), *S. pyogenes* (LMG 15868) and *K. oxytoca* (LMG 3055) were cultured in brain–heart infusion medium (Sigma Aldrich) at 37 °C. *L. salivarius* (LMG 9477), *L. oris* (LMG 9848) and *L. plantarum* (LMG 9211) were cultured in Man, Rogosa and Sharpe medium (Oxoid) at 37 °C. *N. mucosa* (LMG 5136) was cultured in heart infusion medium (Sigma Aldrich) at 33 °C. *P. aeruginosa* (LMG 10639) in medium containing 0.45 g KH₂PO₄, 0.1 g KH₂PO₄, 2.39 g Na₂HPO₄, 1.2H₂O, 1 g (Carl Roth), 1 g Lab-Lemco beef extract and 1 g (Oxoid), 2 g yeast extract 1× (Oxoid), 5 g peptone 1× (Oxoid) and 5 g NaCl 1× (Carl Roth) at 37 °C. *F. nucleatum* (ATCC 10953) was cultured in anaerobic brain–heart infusion medium (Sigma Aldrich) at 37 °C. Except for *F. nucleatum*, all strains were cultured under aerobic conditions. *L. plantarum* was cultured both aerobically and anaerobically.

For the assays in anaerobic conditions, all manipulations and measurement of growth kinetics were performed in an anaerobic workstation (GP-Campus; Jacomet, TCPS NV) under a N₂:CO₂ (90:10, v/v) atmosphere. The preparation of anaerobic medium was done in Balch tubes (Gläserätebau).

Before the start of each experiment, fresh bacteria derived from a –80 °C glycerol stock were plated onto an agar plate and incubated overnight. Next, one colony of each test species was transferred into 9 ml broth and incubated under static conditions overnight at its optimal growth temperature. Subsequently, cultures were transferred (10% v/v) into fresh broth and allowed to grow for 20 h. Cultures were then diluted to 10⁴ intact cells ml⁻¹ in fresh medium, as measured by flow cytometry (BD Accuri C6; Becton, Dickinson and Company) according to Van Nevel et al. (2013), before the start of the experiment.

**Growth assays.** Continuous growth curves were generated in 96-well plates (transparent, flat bottom; Cell Star; Greiner Bio One). In each well, a microbial suspension of 200 µl of each species (10⁴ cells ml⁻¹) was treated with different concentrations of 5-FU (0.1–50 µM) and allowed to grow under static conditions at the optimal growth temperature for each species. Growth was monitored spectrophotometrically (optical density at 620 nm) by means of a Tecan Sunrise plate reader (Tecan) every 30 or 60 min over a period of 18–75 h, depending on the strain. For experiments including uracil, the same experimental set-up was used, but growth was monitored by a Tecan Infinite M200 Pro (Tecan). By fitting the logistic growth model to the growth data, growth rate (µ) and maximal optical density (OD₅₆₂) were calculated using Grofit (version 1.1.1–1) in R (version 3.0.2) (Kahn et al., 2010). The estimation and biological errors were propagated under the assumption of normal distributed and independent errors. Each condition was performed in triplicate or quadruplicate.

**Viability assays.** In each well of a 96-well plate, 200 µl of a diluted bacterial suspension (10⁴ cells ml⁻¹) (see ‘Growth assays’) were treated with different concentrations of 5-FU (0.1–50 µM) for 24 h at the optimal growth temperature of each test species. After incubation, the number of intact and damaged cells was measured by flow cytometry as described by Van Nevel et al. (2013). For this, the samples were diluted in a filter sterile phosphate-buffered solution to obtain cell numbers within the detection range (10⁵–10⁶ cells ml⁻¹). Next, the samples were stained with SYBR Green I (10 000× diluted from stock; Invitrogen) and propidium iodide (final concentration 4 µM; Invitrogen) and incubated for 1 min at 37 °C before measurement. The flow cytometer (BD Accuri C6 flow cytometer; BD) was equipped with a 488 nm solid-state laser, and Milli-Q was used as sheath fluid. Signals were detected in fluorescent channels FL1 (green) and FL3 (red), respectively, equipped with a 518–548 nm and 670 nm bandpass filter. Cell counts were done by measuring the number of particles in a set volume after gating on green vs red fluorescence plots in the BD CSampler software. Quality control of absolute cell counting was done with standardized beads. Background was monitored by measuring a filtered sample, equally diluted as the test samples. Each condition was performed in triplicate or quadruplicate.
**Statistical analysis.** Mixed-model regression of growth rate (µ), OD_{max} and flow cytometer data (log cells ml^{-1}) was performed in the R (version 3.0.2) statistical environment with the concentration as categorical predictor. A random intercept effect was incorporated for each replicate measurement. In order to make correct statistical inference, all models were evaluated for normally distributed residuals with homogeneous variance, by Shapiro–Wilk test (P>0.05) and visually by Q–Q plots. Model parameters were estimated by maximum likelihood. When a significant concentration effect was present (ANOVA, P<0.01), the categories were compared pairwise by post hoc analysis using Tukey’s honest significant difference (HSD) method. All tested concentrations were compared with the control condition (0 µM), and differences were considered significant at P<0.05.

**RESULTS**

**Effect of 5-FU on growth of oral monocultures**

To investigate the effect of 5-FU on the growth of different oral monocultures, growth curves, based on optical density, were generated (see Figs S1 and S2, available in the online Supplementary Material), and growth rates (µ) and OD_{max} were calculated in the presence or absence of different concentrations of 5-FU (0.1–50 µM). 5-FU had no or only a minor inhibitory effect on the growth of *L. salivarius*, *S. mitis*, *S. oralis*, *K. oxytoca* and *P. aeruginosa* (Figs 1 and S1). *S. salivarius* and *P. aeruginosa* did not show a response in terms of OD_{max}. For *S. salivarius*, the growth rate decreased significantly at concentrations of 5-FU higher than 6.3 µM (48–81%). For *P. aeruginosa*, a significant decrease in growth rates was observed at 3.1 µM 5-FU (15–58%). *S. mitis* and *S. oralis* showed only a significant decrease in OD_{max} (67 and 64%, respectively) at the highest tested concentration (50 µM 5-FU) and a decrease in growth rate starting from 6.3 µM (38–75%) and 12.5 µM (32–64%), respectively. *K. oxytoca* showed a significant decrease in OD_{max} (30–35%) for 12.5–50 µM; for growth rates, only a significant decrease was seen at 6.3 µM (37%) but not at higher concentrations. For *L. salivarius*, there was distinct variability in the data, but a significant decrease in growth rate was seen at 6.3 µM 5-FU (75%) and a decrease of 53–57% (not significant) was seen at 12.5–25 µM 5-FU.

*L. oris*, *L. plantarum* (aerobic/anaerobic), *S. pyogenes*, *F. nucleatum* and *N. mucosa* appeared to be more sensitive to 5-FU (Figs 2 and S2). For *L. oris*, a significant decrease in OD_{max} (22–32%) was seen for concentrations ranging from 0.8 to 12.5 µM 5-FU, whereas at 50 µM, no growth was detected. For *L. plantarum* (aerobic), only a significant decrease in growth rate was seen at concentrations higher than 3.1 µM (57–70%), although decreases of 19–41% (non-significant) were seen at 0.4–1.6 µM. *L. plantarum* (anaerobic) showed significant decrease for all tested concentrations (0.1–25 µM) for both growth rate as well as OD_{max}. No growth was detected for concentrations starting from 6.3 µM. *S. pyogenes* did not show a response in terms of OD_{max} but a significant decrease in growth rate at concentrations higher than 0.4 µM 5-FU (33–72%) was observed. For *F. nucleatum*, a significant decrease in OD_{max} and growth rate was seen for 0.8 and 0.4 µM, respectively, and no growth was detected for concentrations higher than 1.6 µM 5-FU. For *N. mucosa*, significant decreases in OD_{max} and growth rate were detected for concentrations higher than 0.8 and 0.2 µM, respectively, and no growth was detected above 12.5 µM 5-FU.

**Effect of 5-FU on viability of oral monocultures**

After 24 h of treatment, the monocultures were stained with SYBR Green/propidium iodide to evaluate the effect of different physiological concentrations of 5-FU on cell viability. SYBR Green penetrates all cells and results in a green fluorescence, whereas propidium iodide penetrates only cells with a damaged cell membrane. Fluorescence resonance energy transfer between propidium iodide and SYBR Green thus only occurs in damaged cells and generates a distinct, lower energy fluorescence signal for the damaged cells. Gating based on this technique makes it possible to quantify both intact and damaged cells in each sample. We used the intact cell densities as an indicator of cell viability (Berney et al., 2007).

Since the number of damaged cells of all tested monocultures (except for *L. salivarius*) was lower than the background, these data are not presented in the graphs. Some species showed only a minor decrease in the number of viable cells (Fig. 3). For *K. oxytoca*, *S. salivarius*, *S. oralis* and *P. aeruginosa*, the number of viable cells decreased with less than 0.65, 1.19, 1.03 and 0.65 log_{10} units, respectively, at a concentration range of 12.5–50 µM. Despite the high variability in the counts of *S. mitis* cells, numbers generally did not drop more than 2.4 log_{10} units or below the background value. The number of intact cells for *L. salivarius* decreased with 0.8 log_{10} unit at 25 µM, coinciding with an increase of damaged cells with 1.6 log_{10} units starting from 3.1 µM.

The most sensitive test species appeared to be *F. nucleatum*, *L. oris*, *L. plantarum*, *N. mucosa* and *S. pyogenes* (Fig. 4). For *L. oris* and *N. mucosa*, there was a decrease in viable cells above 1.6 µM and going below the background at 25 and 6.3 µM, respectively. *L. plantarum* (aerobic/anaerobic), *S. pyogenes* and *F. nucleatum* were even more sensitive and showed a decrease for concentrations higher than 0.2, 0.4, 0.4 and 0.4 µM 5-FU, respectively, and going below the background at 0.4, 0.8, 0.4 and 0.8 µM, respectively.

**Effect of 5-FU on growth of oral monocultures in presence of uracil**

To investigate the role of DPD in the sensitivity response towards 5-FU, growth experiments were performed with two resistant species, namely, *S. salivarius* and *K. oxytoca*, in the presence and absence of uracil, an inhibitor of DPD. DPD is normally involved in the degradation of 5-FU into the non-toxic dihydrofluorouracil. Quantification of 5-FU and dihydrofluorouracil was not possible by HPLC analysis, owing to high background (data not shown). Hence, we hypothesize that 5-FU-resistant microorganisms are able to incorporate dihydrofluorouracil into nucleic acids.
**Fig. 1.** 5-FU has only a minor negative effect on the growth of *K. oxytoca, S. salivarius, S. mitis, S. oralis, P. aeruginosa* and *L. salivarius*. Maximal optical density (OD\(_{\text{max}}\)) and growth rate (\(\mu\)), calculated from growth curves of oral monocultures treated with different concentrations of 5-FU (0.1–50 \(\mu\)M (AV ± SD; solid line = control condition (0 \(\mu\)M)). Significant deviations from the control condition (0 \(\mu\)M) are indicated by asterisks (*P<0.05*).

**Fig. 2.** 5-FU has a major negative effect on the growth of *L. oris, L. plantarum* (aerobic/anaerobic), *S. pyogenes, F. nucleatum* and *N. mucosa*. Maximal optical density (OD\(_{\text{max}}\)) and growth rate (\(\mu\)), calculated from growth curves of oral monocultures treated with different concentrations of 5-FU (0.1–50 \(\mu\)M (AV ± SD; solid line = control condition (0 \(\mu\)M)). Significant deviations from the control condition (0 \(\mu\)M) are indicated by asterisks (*P<0.05*).
organisms might become more sensitive to 5-FU in the presence of uracil, a competitive substrate of DPD, provided that the activity of 5-FU is DPD mediated. Therefore, growth curves were generated for 0, 1.6 and 12.5 µM of 5-FU with or without uracil (5-FU : uracil ratio of 1 : 4) (similar results were obtained with a 1 : 10 ratio; data not shown). At 1.6 µM 5-FU, uracil did not modulate the resistance of either test species towards 5-FU (data not shown). Remarkably, at 12.5 µM, uracil stimulated growth in the presence of 5-FU, rejecting our hypothesis that DPD is involved in microbial resistance towards 5-FU (Fig. 5; P-values see Tables S1 and S2).

**DISCUSSION**

5-FU is one of the oldest chemotherapeutic agents and causes multiple side effects such as oral mucositis. It is known to have an antibacterial effect at high concentrations, but its effect at physiologically relevant concentrations is still under-explored. In this study, we show that, at low concentrations (0.1–50 µM), there is a great variability in 5-FU sensitivity among oral micro-organisms. The combination of growth and viability test results suggests that oral micro-organisms can be divided into two sensitivity groups: the resistant micro-organisms and the sensitive micro-organisms. The first group consists of *K. oxytoca*, *S. salivarius*, *S. mitis*, *S. oralis* and *P. aeruginosa*, whereas *L. salivarius* had more damaged cells after treatment with 3.1–50 µM 5-FU. Flow cytometric analysis of viability of oral monocultures treated with different concentrations of 5-FU (AV ± sd). Significant deviations from the control condition (0 µM) are indicated by asterisks (P<0.05).
P. aeruginosa, which only experience a minor decrease in growth and viability following exposure to high concentrations of 5-FU (12.5–50 µM). The second group comprises L. oris, L. plantarum (aerobic/anaerobic), L. salivarius, S. pyogenes, N. mucosa and F. nucleatum, which appear to be more sensitive to 5-FU with clearly negative effects on both growth and viability starting from 0.4 to 3.1 µM. One of the sensitive species is somewhat singular, namely, L. salivarius for which there is a distinct increase in damaged cells at higher concentrations of 5-FU, as measured by flow cytometry.

Our finding of differential microbial sensitivity to 5-FU correlates with previous in vivo observations. In a rat mucositis study, Stringer et al. (2009) identified P. aeruginosa and Escherichia coli as 5-FU-resistant species. Furthermore, P. aeruginosa and E. coli were shown to be able to overgrow the oral

---

**Fig. 4.** 5-FU causes a major decrease in viable cell counts starting from 0.4 to 1.6 µM for L. oris, L. plantarum (aerobic/anaerobic), S. pyogenes, F. nucleatum and N. mucosa. Flow cytometric analysis of viability of oral monocultures treated with different concentrations of 5-FU (AV ± SD). Significant deviations from the control condition (0 µM) are indicated by asterisks (P<0.05).
community and penetrate the damaged underlying mucosa thereby causing local infections (Stringer et al., 2009). Another study with rats reported similar shifts, with Gram-negative rods becoming more abundant upon treatment with 5-FU (Von Bültzingslöwen et al., 2003). Also, other chemotherapeutics have been shown to generate differential microbial response to 5-FU leading to infections, as demonstrated for pathogenic *P. aeruginosa* in leukaemic patients (Goldschmidt & Bodey, 1972). Therefore, microbial 5-FU resistance should be considered as an important risk factor for infections especially in the context of oral mucositis.

Previous studies investigating the antibacterial properties of 5-FU primarily evaluated concentrations that are not representative of *in vivo* 5-FU concentrations during chemotherapy. Plasma concentrations after continuous infusion range from 0.1 to 8.8 µM, depending on the dose (300–2300 mg·m⁻²·day⁻¹) (Grem, 2000). In the mouth, saliva levels during continuous infusion range from 0.08 to 0.8 µM (Joulia et al., 1999), hence significantly lower than what can be measured in plasma but still high enough to affect the most sensitive micro-organisms in the mouth. Furthermore, plasma concentrations can reach much higher concentrations in DPD-deficient patients due to the tenfold longer half-life time of 5-FU (Saif et al., 2009).

In humans, DPD seems to play an important role in obtaining resistance to 5-FU, since it can break down 5-FU to the non-toxic dihydrofluorouracil (Aziz et al., 2013). Literature data on microbial DPD are scarce, but the presence of DPD activity has previously been shown for *P. aeruginosa* (Kim & West, 1991) and *E. coli* (West, 1998). In our study, *K. oxytoca*, *S. salivarius*, *S. mitis*, *S. oralis* and *P. aeruginosa* were shown to be 5-FU resistant. However, a search of the NCBI protein database on all tested micro-organisms indicated that, theoretically, only *P. aeruginosa* and *L. oris* contain a DPD enzyme. Hence, no clear link between microbial DPD and 5-FU sensitivity could be identified. In clinical settings, a combination of 5-FU and uracil, an inhibitor of DPD, is used to improve the efficiency of 5-FU. Uracil competitively

![Fig. 5. At 12.5 µM, 5-FU had a small negative effect on the growth of *P. aeruginosa* and *S. salivarius*, and by adding uracil, they both became somewhat more resistant to 5-FU. Maximal optical density (ODmax) and growth rate (µ) calculated from growth curves of *P. aeruginosa* and *S. salivarius* treated with or without 5-FU (12.5 µM) and with or without uracil (50 µM) (AV ± SD). Relevant significant deviations are indicated by asterisks (P<0.05).](image-url)

http://jmm.microbiologyresearch.org 781
binds to DPD, reducing the degradation of 5-FU to dihydro-fluorouracil (Takechi et al., 1997; Omura, 2003). In our study, we applied the same principle to test whether microbial 5-FU sensitivity is dependent on the presence of DPD in resistant micro-organisms, yet our results suggest that DPD is not a key enzyme.

Many other enzymes have been reported to be involved in the pharmacokinetics of 5-FU, including thymidylate synthase (TS). TS provides the sole de novo production of thymidylate and is inhibited by 5-FU (Longley et al., 2003). In cell lines, a positive relationship exists between DPD and TS levels and 5-FU sensitivity (Beck et al., 1994). In Caenorhabditis elegans, overexpression of TS and DPD led to increased survival after treatment with 5-FU (Kim et al., 2008). Also, in humans, overproduction of TS resulted in 5-FU resistance (Clark et al., 1987; Johnston et al., 1995). Interestingly, a search for TS in the NCBI protein database in our study showed hits for all tested species. Therefore, TS and other enzymes may play a role in 5-FU resistance. At least for the treatment of colorectal cancer patients, the analysis of three predictive markers (TS, DPD and thymidine phosphorylase) is used to predict 5-FU efficacy (Salonga et al., 2000).

In conclusion, our study shows that 5-FU sensitivity varies among different oral microorganisms and that two clear groups can be distinguished: resistant and sensitive microorganisms. Some species, such as L. oris, L. plantarum, L. salivarius, S. pyogenes, N. mucosa and F. nucleatum, are sensitive at concentrations as low as 0.4 μM, which can be measured in blood and saliva during continuous 5-FU treatment. We also provided evidence that the DPD enzyme is probably not responsible for microbial resistance to 5-FU. Our data further indicate that oral mucositis patients are likely to develop infections caused by an overgrowth of 5-FU-resistant strains, such as the multi-drug-resistant P. aeruginosa, and dysbiosis of the mucosa. To get a complete picture of the impact of 5-FU on the oral microbiome, an ecosystemic approach is needed in further research.

ACKNOWLEDGEMENTS

Eline Vanlancker is a doctoral research fellow supported by the Bijzonder Onderzoeksfonds (Ghent University; B/13807/01-BOF13/DOC/280). Barbara Vanhoecke was funded by the Seventh Framework Programme (FP7/2011) under grant agreement number 299169 (Mucositis Platform). The authors would like to thank Jan Arends and Kim De Paepe for their review of the manuscript.

REFERENCES


