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Abstract. Palmoplantar erythrodysesthesia (PPE), or hand-foot syndrome, is a cutaneous toxicity under various chemotherapeutics contributing to the most frequent side effects in patients treated with capecitabine (Xeloda[®]). The pathomechanism of PPE has been unclear. Here, the topical detection of capecitabine in the skin after oral application was shown in 10 patients receiving 2500 mg/m²/day capecitabine. Sweat samples were taken prior to and one week after oral administration of capecitabine. Using high-resolution continuum source absorption spectrometry, the changes in concentrations of fluorine, which is an ingredient of capecitabine, were quantified and statistically analyzed. Here, we show an increase in fluorine concentrations from 40 ± 10 ppb (2 ± 0.5 pM) before capecitabine administration to 27.7 ± 11.8 ppm (14.6 ± 6.5 nM) after application, $p < 0.001$. The results show the secretion of capecitabine on the skin surface after oral administration, indicating a local toxic effect as a possible pathomechanism of PPE. © 2016 Society of Photo-Optical Instrumentation Engineers (SPIE) [DOI: 10.1117/1.JBO.21.4.047002]

Keywords: high-resolution continuum source absorption spectrometry; atomic absorption spectroscopy; sweat analysis; hand and foot syndrome; chemotherapy; inverse penetration.

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1 Introduction

Today there is a variety of highly effective chemotherapeutic agents providing a therapeutic option to fight tumor cells. However, these chemotherapeutic agents are also known to induce a whole range of cutaneous side effects. Cutaneous side effects often arise in the form of erythematous skin symptoms, pruritic rashes, and sores and open wounds. A typical appearance of dermal side effects is hand-foot syndrome, or palmoplantar erythrodysesthesia (PPE), which occurs, in particular, under treatment with certain chemotherapeutic agents, such as pegylated liposomal doxorubicin (Caelyx[®]), capecitabine (Xeloda[®]), docetaxel, sorafenib (Nexavar[®]), or sunitinib (Sutent[®]).^{1–3} The severity and onset of skin symptoms show a high interindividual variability and also depend on the chemotherapeutic agent and dosage. Once these skin lesions occur, the treatment of severe PPE symptoms can be difficult.⁴ Dose modification, postponement, or even discontinuation of chemotherapy⁵ can be necessary since dermatological treatments, e.g., with anti-inflammatory agents or topical cooling applications, show no satisfactory therapeutic effects.^{2,4} Hand-foot syndrome is characterized by inflammatory skin lesions ranging from erythema, edema, and erosions to rha-gades, blisters, and ulcerations accompanied by paresthesia, dysesthesia, or even severe pain.^{2,5} The described skin lesions often considerably impair the quality of life and daily life activities in affected patients.⁶ Therefore, there is a strong need to apply and improve preventive and supportive measures against

dermal side effects under chemotherapy. A range of anticancer drugs is detectable depending on their optical properties.^{7,8} Due to the fluorescent properties of the chemotherapeutic drug doxorubicin, pegylated liposomal doxorubicin (Caelyx[®]) can be cutaneously detected by fluorescence spectroscopic measurements. It was shown that Caelyx[®] was detectable on the skin surface being secreted by the sweat glands.^{9,10} It evenly spreads on the skin surface, penetrating into the upper epidermis, where it shows its cumulative toxic effects by forming free radicals that damage the cellular structures. An effective preventive and therapeutic strategy was developed in previous studies for Caelyx[®] patients by the topical application of an antioxidant-containing ointment.^{4,11} Due to the regular topical application of this ointment, a consistent protective layer is formed, which prevents the chemotherapeutic agent from penetrating into the skin and simultaneously neutralizes the chemotherapy-induced free radicals using antioxidants before they develop the damaging effects to the skin cells. In addition to that, there are other chemotherapeutic agents that show similar cutaneous adverse reactions after systemic application. Until now, the local secretion of chemotherapeutic agents on the skin has not been detectable for chemotherapeutic agents showing no fluorescence signals. Detection would be essential to examine both potential pathogenic mechanisms and therapeutic strategies. A particular substance in this regard is the chemotherapeutic agent capecitabine (Xeloda[®]), causing dermal side effects in ~50% of treated patients.^{5,6,12} Capecitabine is an orally administered drug, which is converted to active 5-fluorouracil by enzymatic processes. Due to differences in enzyme activity

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between healthy and cancerous cells, capecitabine has a specific and selective effect on tumor cells. It is usually administered at a dosage of 1250 mg/m² twice daily for two weeks followed by a chemotherapy-free interval of one week.⁵

Capecitabine is mostly well tolerated, but PPE is among its most frequent side effects. The exact pathomechanism of capecitabine-induced PPE has remained unclear, as there has been no means to detect capecitabine after systemic application on the skin. There are several theories about the pathomechanism of capecitabine-induced PPE.¹² The accumulation as well as the excretion through the sweat glands has been suspected to cause the cytotoxic effects on skin sites with increased density of sweat glands, such as the palmar and plantar areas.^{2,12} Increased mechanical pressure and heat are also known to trigger and enhance PPE skin lesions under chemotherapy.^{1,12,13} The investigation into whether capecitabine emerges with sweat on the skin surface like doxorubicin is of great importance, since the topical prevention strategy that has been successfully used in Caelyx[®] patients could be transferred to capecitabine. Here, we demonstrate a method for the detection of capecitabine on the skin surface after systemic application. The topical detection of capecitabine is based on the fact that fluorine is an important ingredient of capecitabine, while fluorine in the human body or on the skin is detectable only at very low concentrations. Recently, a highly sensitive atomic absorption spectroscopy (AAS) method was developed, which made it possible to detect fluorine atoms specifically and quantitatively. This method was used in the following study to investigate sweat samples for changes in their fluorine content, the samples having been obtained from the skin surface of patients before and after chemotherapeutic treatment with capecitabine.

2 Materials and Methods

The investigations were performed in 10 cancer patients (eight male and two female patients) aged between 57 and 89 years, who were treated with capecitabine (Xeloda[®]) within their therapeutic schedule. Six patients were diagnosed with metastatic colon cancer, three patients with rectal cancer, and one patient with cecum cancer. Capecitabine was dosed at 1250 mg/m² administered orally twice daily in the morning and evening for a treatment period of two weeks followed by a one-week rest period (q21 days).

All measurements were performed within the week prior to the first cycle of capecitabine and 10 days after the initial treatment day, within the first cycle of chemotherapeutic treatment.

The study had been approved by the ethics committee of the Charité–Universitätsmedizin Berlin and was performed according to the Declaration of Helsinki.

2.1 Collection of Sweat Samples

The sweat samples were collected using the Macroduct[®] sweat collection system (U.S. patent No. 4,542,751), which has been developed and commonly applied for laboratory diagnosis of cystic fibrosis in newborns (Fig. 1).

Within this study, the sweat collection system was placed on the skin surface on the forearm of each patient for about 30 min. In this way, a few tens of microliters of sweat could be collected by the capillary tube even without the recommended pilocarpine iontophoresis. Furthermore, negligible fluorine concentrations of the plastic tube material were analytically confirmed.

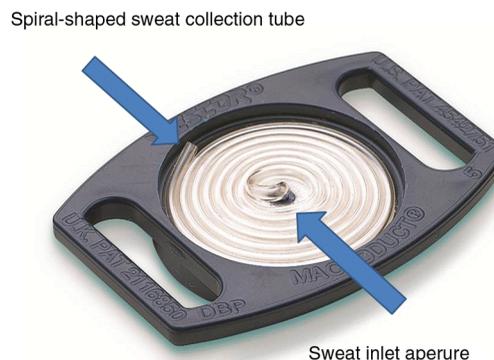


Fig. 1 Macroduct[®] sweat collection system.

2.2 Analysis of Sweat Samples for Their Fluorine Concentration

The individual quantities of the carefully collected sweat samples were introduced directly into a miniaturized graphite furnace. By adequate heating of the furnace, the fluorine concentration was determined by high-resolution continuum source absorption spectrometry (HR-CSAS). General information about this analytical technique and the essential spectroscopic equipment, both developed substantially at Leibniz-Institut für Analytische Wissenschaften–ISAS–e.V., Berlin, Germany, can be found in previous publications.^{14–16}

The HR-CSAS measurements were performed at ISAS, Berlin, using an experimental setup based on a contrAA 600 spectrometer (Analytik Jena AG, Jena, Germany), a miniaturized graphite tube of 2 mm inner diameter (Schunk Graphite Technology, Heuchelheim, Germany), and a unique autosampler for accurate handling of small sample volumes down to 30 nL. The spectrometer allows access to arbitrary wavelength intervals ranging from 190 to 900 nm at high spectral resolution of $\lambda/\Delta\lambda = 1/140,000$ per pixel to investigate single atomic and molecular absorption lines. Before analytical use, the graphite tube was modified with a ZrC coating to increase the sensitivity of the fluorine detection by a factor of about 20. As a continuum source, a 300-W xenon short-arc lamp (XBO 301, GLE, Berlin, Germany) was applied.

Generally, the determination and quantification of fluorine was accomplished by measuring the molecular absorption of CaF molecules. For samples without the additional F-source from the capecitabine administration, the determination was performed at the sensitive CaF line at 606.44 nm. Due to the high F-concentration in the samples with additional F-source after intake of capecitabine, the less sensitive CaF line at 583.069 nm was used for further analysis (Fig. 2). The sweat samples did not need any treatment before the measurement. For sample analysis, 0.33 μ L sweat sample together with 0.2 μ L of 0.5% Ca solution was charged into the graphite tube. The addition of extra Ca was necessary for the formation of the CaF molecule during the stepwise heating procedure. A pyrolysis temperature of 1100°C and a vaporization temperature of 2100°C were employed. A high pyrolysis temperature exceeding 1000°C is necessary for removing the major component NaCl from the sweat sample. Calibration was accomplished using a calibration curve recorded with an aqueous fluorine standard solution made of NaF.

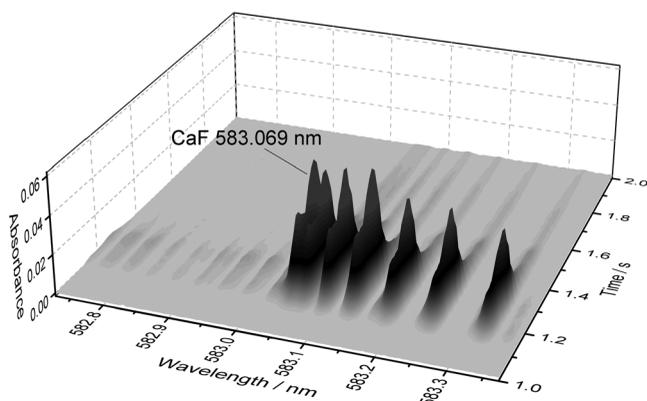


Fig. 2 Time- and wavelength-resolved CaF molecular absorption spectrum around 583.069 nm.

3 Results

All 10 participating patients showed a considerable increase in fluorine concentration in the sweat samples after oral administration of capecitabine compared to basement measurements prior to the first cycle of capecitabine treatment. This increase was highly significant ($p < 0.001$). Before capecitabine administration, the overall fluorine concentrations in the sweat samples from the palms of the patients amounted to 40 ± 10 ppb (2 ± 0.5 pM), which is a concentration that would also be expected in healthy human skin. The second collection of sweat samples of all volunteers 10 days after capecitabine administration showed a substantial increase to a mean fluorine concentration of 27.7 ± 11.8 ppm (14.6 ± 6.5 nM), corresponding to an increase by factors from 300 to 1200 in all sweat samples. Three patients had to discontinue their chemotherapeutic treatment during the study. The sweat samples of these patients showed a considerable decrease in fluorine concentrations after discontinuation of capecitabine administration compared to the initial values measured before chemotherapy.

There was no case of hand-foot syndrome observed during the three-week observational period of the study.

4 Discussion

The topical detection of capecitabine through a change in fluorine concentrations shows that capecitabine is excreted on the skin surface after systemic application. The fact that all subjects within this study showed enhanced fluorine concentrations, while PPE shows a general incidence of $\sim 50\%$ in capecitabine treated patients,^{1,6,12} indicates a dose-dependent effect and the dependency on other influence factors, such as skin barrier disruptions or differences in the metabolization of capecitabine in treated patients. In addition, the individual antioxidant status might be critical for regeneration from toxic effects on the skin cells, as discussed previously.⁴ Furthermore, external mechanical influences, such as pressure or friction on the skin, which were not assessed within this study, might favor the development of skin lesions.¹⁷ The results of this study have proven the excretion of capecitabine on the skin surface, indicating a local toxic effect of capecitabine on the skin cells as a possible pathomechanism of PPE skin lesions, as was shown for anthracycline-associated PPE.^{9,10} A potential protection from the destructive effects of free radical formation in the skin after systemic chemotherapy could be provided by the preventive application of highly concentrated antioxidants.^{4,11}

Topically applied antioxidants could therefore serve as an effective preventive and therapeutic option for capecitabine-induced PPE. Another possible protective measure could be the use of optimized absorbent textile materials containing super-absorbent particles,¹⁸ in order to remove the chemotherapeutic after inverse penetration from the skin surface, eliminating the local toxic effect of the chemotherapeutic agent.

Further investigations will be required to explore the exact pathomechanism of capecitabine-induced PPE and the processes involved. The findings of this study also demonstrate the effectiveness of AAS as a suitable method to detect and quantify the topical concentration of systemically applied substances in sweat from the skin surface.

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Sora Jung studied medicine at Charité–Universitätsmedizin Berlin, Germany, and Université Denis Diderot Paris, France. After a research stay at Dartmouth Medical School and at the World Health Organization headquarters in Geneva, Switzerland, she has been working since 2013 as a resident and research assistant at the CCP, with her main research focus on the interaction of antioxidants and free radicals in the skin, clinical investigations, and preventive strategies of chemotherapy-induced palmar–plantar erythrodysesthesia.