Influence of Chemotherapy on the Antioxidant Status of Human Skin

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Abstract. *Background: Palmoplantar erythrodysesthesia is a frequent dermal side-effect during chemotherapy. Previous investigations showed radical formation subsequent to doxorubicin infusion and preventative and therapeutic effects of an antioxidant-containing ointment. Patients and Methods: Using a non-invasive vivomeasuring system (Biozoom®; Biozoom Services GmbH, Kassel, Germany) changes in the antioxidant status (as measured by relative carotenoid concentration) of the skin prior to and after intravenous administration of paclitaxel, docetaxel and 5-fluorouracil were investigated in 42 patients with cancer. Results: A significant decrease of antioxidant concentration subsequent to intravenous administration was found for all investigated chemotherapeutic agents. The mean concentration of carotenoids decreased from 3.59±1.26 arbitrary units (a.u.) to 3.41±1.28 a.u. ($p<0.001$) after paclitaxel administration, from 6.33±2.43 to 5.63±2.29 a.u. after docetaxel ($p=0.027$) and from 4.26±1.81 to 3.98±1.53 a.u. ($p=0.042$) after 5-fluorouracil infusion. Conclusion: Oxidative stress might play a significant role in the pathomechanism of palmoplantar erythrodysesthesia associated with paclitaxel, docetaxel and 5-fluorouracil. Therefore, an antioxidant-containing ointment might serve as preventative and therapeutic option.*

Developed in the early 20th century, chemotherapy is an effective way to fight cancer cells using a variety of potent chemotherapeutic agents (1). These treatments have specific effects on arresting the progression of tumor cells by acting in various stages of the cell cycle (2, 3). Antimetabolites, such as 5-fluorouracil (5-FU), hamper the synthesis of DNA or RNA either by inhibiting the production of normal purine or pyrimidine precursors, or by interfering with them (3). Anthracyclines, such as doxorubicin, not only intercalate into DNA, but also produce free radicals causing single-strand breaks in DNA (3).

Although anticancer therapy is targeted to eliminate cancer cells, there exists a variety of side-effects affecting healthy cells of other organs. A frequent dermal side-effect due to chemotherapeutic treatment is hand–foot syndrome, also known as palmar-plantar erythrodysesthesia (PPE). This is a common dose-limiting adverse effect under treatment with several agents, such as pegylated liposomal doxorubicin (PLD), 5-FU, capecitabine, docetaxel, or tyrosine-kinase inhibitors such as sorafenib and sunitinib (4-6). The initial symptoms are swelling or erythema in the palmar and interdigital areas of the hands and soles of the feet, accompanied by dysesthesia and paresthesia, which can progress to burning pain with dryness, rhagades, desquamation, ulceration, edema and rash (5). PPE is rarely life-threatening, but it can significantly impair the quality of life of patients, leading to the need for dose reduction or interruption of therapy (6). Depending on the severity, there are three grades of PPE according to the National Cancer Institute (Table I).

The exact pathomechanisms of PPE are unclear for most chemotherapeutic agents and treatment options are very limited. Martschick *et al.* showed that systemically applied PLD, one of the most widely used antitumor agents, is secreted with sweat onto the skin surface, spreading over the skin and penetrating into the stratum corneum as if topically applied (7). Using *in vivo* laser scanning microscopy, PLD was detected on the skin surface about an hour after

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Table I. *Classification of palmar-plantar erythrodysesthesia (25).*

Grade	Definition
1	Minimal skin changes (*e.g.* erythema, swelling, or hypokeratosis) without pain
2	Skin changes (*e.g.* peeling, blisters, bleeding, edema, or hyperkeratosis) with pain; limiting instrumental ADL*
3	Severe skin changes (*e.g.* peeling, blisters, bleeding, edema, or hyperkeratosis) with pain; limiting self care ADL**

ADL: Activities of daily living; *Instrumental ADL refers to preparing meals, shopping for groceries or clothes, using the telephone, managing money, *etc.*; **Self care ADL refers to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not being bedridden.

intravenous infusion. Doxorubicin exerts its cytotoxic effect primarily through interactions with DNA. In addition, doxorubicin interacts with molecular oxygen, producing reactive oxygen species (ROS) and causes single-strand breaks in DNA (3). It seems that the effects of PLD, both on the tumor and on the skin, are similar, explaining a possible mechanism of doxorubicin-induced PPE.

Free radicals can damage cells and cell compartments (8). The fact that PPE occurs mainly on the palms and soles is due to the anatomical difference between these areas and other skin sites, namely the increased thickness of the stratum corneum and the high density of eccrine sweat glands. It was discussed that the secretion of doxorubicin with sweat after intravenous administration subsequently induced the formation of free radicals in the epidermal skin layers, reducing the antioxidant potential of the skin. Antioxidant substances such as carotenoids (beta-carotene, lycopene, and lutein/zeaxanthin), vitamins (A, C, D, and E), enzymes (superoxide dismutase, catalase, and glutathione peroxidase), as well as various other substances (flavonoids, lipoid acid, uric acid, selenium, coenzyme Q10), are very efficient in neutralizing free radicals and especially ROS (9-11). Based on these mechanisms, a preventative and therapeutic strategy for PPE has been developed using topical application of antioxidant-containing ointment. In previous investigations, it was demonstrated that the regular topical application of this antioxidant-containing ointment had efficient preventative and even therapeutic effects on doxorubicin-induced PPE (12, 13).

In order to enhance the understanding of PPE pathogenesis and preventative options, the present study investigated the change in antioxidant status of the skin after systemic administration of paclitaxel, docetaxel, or 5-FU. We hypothesized that a decline in the antioxidant status after systemic administration of these chemotherapeutic agents would indicate a similar pathomechanism to that of doxorubicin-induced PPE.

Materials and Methods

Patients. Patients were recruited at the Department of Hematology and Oncology and the Department of Gynecology of the Charité University of Medicine Berlin. The included patients suffered from primary and metastatic breast or ovarian cancer, oral, nasopharyngeal, pancreatic or colorectal cancer, or neuroendocrine tumors. Prior to the beginning of the investigation, the study had been approved by the Ethics Committee (approval number EA1/235/14) of the Charité University of Medicine Berlin. The investigations were conducted in accordance with the ethical guidelines of the Declaration of Helsinki. All participants gave their informed written consent. Volunteers were only included if they showed neither any pathological skin lesion nor initial symptoms of PPE, such as swelling, erythema, dysesthesia or paresthesia on their hands and feet at the time of the measurements.

Chemotherapeutic agents. The chemotherapeutics investigated in this study included docetaxel, paclitaxel and 5-FU. The chemotherapeutic agents investigated in our study can be classified into different groups regarding their mechanism of action. In previous studies, we observed that doxorubicin leads to radical formation and thus to a decline in the antioxidant status of the skin (7, 14). The majority of patients in our study received paclitaxel, which has high activity against ovarian and breast cancer (3). By binding to tubulin molecules, it stabilizes these excessively so that depolymerization in mitosis does not occur, which leads to cell death. Docetaxel is a taxane having a similar mechanism to paclitaxel, but with slightly different side-effects (2, 3). The third chemotherapeutic agent applied in our investigation was 5-FU, a pyrimidine analog. It impedes the conversion of deoxyuridylic acid to thymidylic acid. Without thymidine, DNA synthesis decreases, which leads to imbalanced cell growth and cell death.

Each treatment was systemically applied by intravenous infusion. Paclitaxel was administered at a dose of 60-90 mg/m^2 weekly or 175 mg/m^2 every 3 weeks, while the patients treated with docetaxel had their infusion at a dose of 30-35 mg/m^2 weekly or 75-100 mg/m^2 every 3 weeks. 5-FU was administered at a dose of 2,000 mg/m^2 weekly or 2,400 mg/m^2 every 2 weeks.

Study design. The concentration of carotenoids, serving as markers of the overall antioxidant status of the skin, was non-invasively measured immediately before and directly after termination of intravenous administration of chemotherapy using a miniaturized measuring system (Biozoom® sensor; Biozoom Services GmbH, Kassel, Germanya; see below). Measurement was made in each patient once before and once after chemotherapy administration during the scheduled treatment period. Regarding paclitaxel and docetaxel infusions, the time period between the first and the second measurement was 1.5 to 2 h. Depending on the individual therapy regimen for each patient, 5-FU was administered over a period of 22 to 46 h. Therefore, measurements of the antioxidant status in

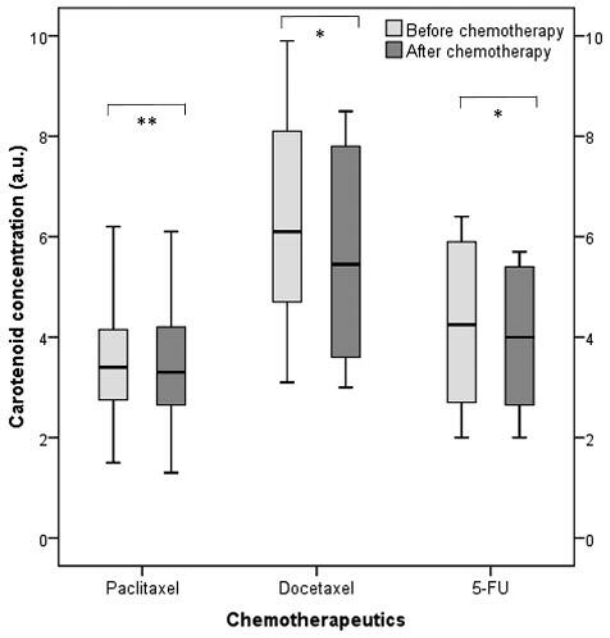

Figure 1. *Boxplot of carotenoid concentrations according to chemotherapeutic agent. Whiskers represent the minimum and the maximum concentrations within the 1.5-fold interquartile range. *p<0.05; **p<0.01.*

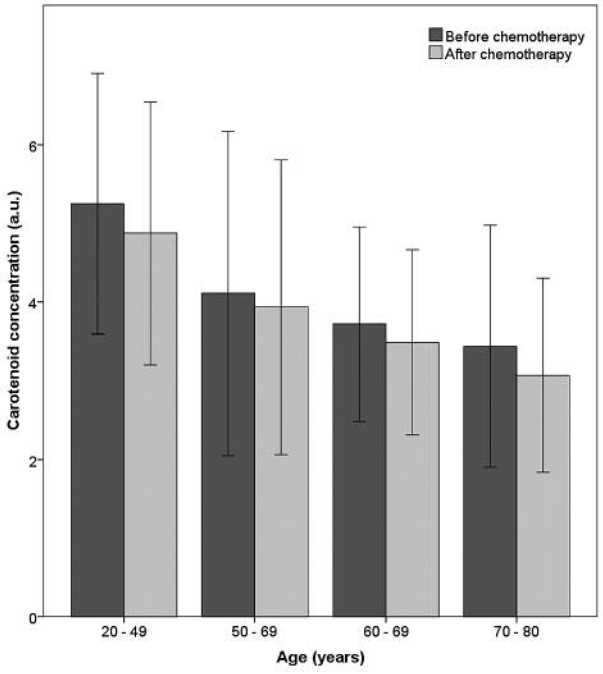

Figure 2. *The chart shows the mean carotenoid concentrations before and after chemotherapy administration in different age groups. Whiskers represent the standard deviation.*

these patients were conducted before infusion and 22 to 46 h afterwards.

Overall, the investigations were conducted over the course of 24 months.

Noninvasive determination of antioxidants in the skin. In previous studies, it could be demonstrated that carotenoids can be measured as a marker substances for the whole antioxidant status of the human skin (15). Using a Biozoom® sensor, whose working principle is based on reflection spectroscopy, the concentration of carotenoids in human skin was determined *in vivo*and noninvasively (16, 17). The measuring device has been described in detail previously (15). Briefly, the device is a mobile measuring system with a light emitting diode (LED) operating at a wavelength of 465±25 nm as a source of excitation. This wavelength was chosen due to the absorption maximum of carotenoids in this range. The bottom of the skin scanner contains a measuring head carrying an optical window. The LED emits a ray of light through this channel window which is brought into contact with the skin. The design of the channel window requires tight contact between the skin and the measuring head to exclude the undesirable influence of light from the outside. This optical window not only sends light out to excite the tissue below the surface of the skin, but also receives the light reflected back from the tissue. The signal reflected from the human skin was analyzed by a miniature replicated holographic grating spectrometer (15, 18). Measured carotenoid concentrations were displayed on a linear scale from 1 to 12 in arbitrary units (a.u.).

The measurement of carotenoids was performed twice on the thenar eminence of each patient's palms: once prior to the systemic application of chemotherapeutic agent and once after termination of the intravenous drug administration. Each measurement took about 60 seconds. For every measurement, both left and right palms were measured twice each to determine the mean carotenoid concentration. Along with the measurements, the patients were asked about the occurrence of dermatological side-effects under treatment with the chemotherapeutic agents. Their skin status, including skin lesions and conditions such as dryness, wounds or ulcerations, was also examined prior to making the measurements.

Statistical analysis. The statistical analysis was conducted using SPSS version 22 for Windows (IBM Corporation, Armonk, NY, USA). The Kolmogorov–Smirnov test was applied to test for normal distribution of continuous data. All described data were analyzed using nonparametric testing. The Wilcoxon signed-rank test was used to analyze differences in the antioxidant status before and after treatment. *p*-Values of less than 0.05 were considered to indicate statistical significance.

Results

A total of 42 patients, aged from 27 to 77 years, were enrolled, including 28 patients treated with 60-175 mg/m^2 paclitaxel, six treated with 30-100 mg/m^2 docetaxel, and eight treated with 2000-2400 mg/m^2 5-FU.

Influence of chemotherapeutic agents on carotenoid concentration. The carotenoid concentration before the

application of the chemotherapeutic agents showed a high variance, ranging between 1.5 and 9.9 a.u. The mean carotenoid concentration prior to the infusion of the chemotherapy in all patients was measured at 4.08±1.77 a.u., while it decreased to 3.81±1.63 a.u. after infusion; this difference was found to be statistically highly significant (p=0.000017).

Influence of the different chemotherapeutic agents. All chemotherapeutic agents significantly reduced the antioxidant status.

The mean concentration of carotenoids before the application of paclitaxel was 3.59±1.26 a.u. After infusion, the mean concentration was significantly reduced to 3.41±1.28 a.u. (p=0.000231). Patients who received docetaxel showed a significant decline of their antioxidant status, from a mean value of 6.33±2.43 to 5.63±2.29 a.u. (p=0.027). 5-FU also significantly reduced the carotenoid concentration: the mean antioxidant level decreased from 4.26±1.81 to 3.98±1.53 a.u. (p=0.042). Figure 1 presents carotenoid concentration depending on the chemotherapeutic drug.

Further observations. Prior to the study it was expected that patients with cancer in general would have a notably lower antioxidant concentration than shown in this study, as they suffer from a disease that can cause psychological and physical distress. Surprisingly, the concentration of carotenoids varied considerably between individual patients and showed a high overall variance. The patients who had higher antioxidant levels reported that they tried to eat and live healthily, avoiding stress exposure and consuming high amounts of antioxidant-rich fruit and vegetables.

Although no significant differences were found between different age groups of patients, Figure 2 shows that the group of younger patients had a tendency to have a higher mean level of carotenoids. Furthermore, there was no significant difference found in the antioxidant status depending on the type of cancer nor on gender.

Discussion

Influence of the antioxidant status. The antioxidant status of the human skin correlates with antioxidant concentrations measured in the blood circulation (19-21). Systemically applied substances can reach the papillary dermis by blood circulation and can be secreted on the skin surface *via* the sweat glands. This can account for the significant influence of intravenously administered chemotherapeutics on the antioxidant status of the skin. Furthermore, there is an individual variance due to lifestyle factors, living environments, as well as medication and disease. All influencing factors form an equilibrium between antioxidants and free radicals in the human body. Many antioxidants cannot be synthesized by humans, but have to be taken-up in healthy nutrition, especially fruits and vegetables. Therefore, the antioxidant status of individuals depends on many factors, among them a healthy diet (22). However, not only nutritional habits but also smoking and alcohol consumption influence the antioxidant status (21). Previous investigations showed that low carotenoid values correlate with physical stress factors, such as illness and sleep deprivation, as well as psychological stress and excessive physical activity, which can cause their rapid decline (23). Since the measurements of this study were conducted before and directly after chemotherapy administration, it is possible that psychological distress due to the clinical setting and medication process enhanced the decrease of carotenoids.

PPE association with chemotherapeutic agents. There are many case reports and studies with patients affected by PPE as a side-effect of chemotherapeutic agents such as doxorubicin, paclitaxel, docetaxel and 5-FU (4, 24). Despite the differences in the investigated chemotherapeutic drugs and doxorubicin in their mechanisms, the carotenoid concentration in the skin declined significantly after administration of all investigated agents. Skin toxicity can effect dose density, with treatment delay, dose reduction and discontinuation, and influence the quality of life of the patients. These can negatively influence the clinical outcome and tumor control rate.

The cytotoxic effect of doxorubicin is based on free radical processes, which are also assumed to cause the inflammatory and necrotic skin lesions of PPE. This study revealed subsequent radical formation after infusion of the PPE-associated chemotherapeutics paclitaxel, docetaxel and 5-FU. This finding is interesting, since the main cytotoxic effects of these chemotherapeutic agents are not attributed to radical formation. Although the measured decline in carotenoids after infusion of the investigated chemotherapeutics, especially paclitaxel, was relatively small, a cumulative toxic effect can be presumed after several infusions of chemotherapy. On the other hand, the small effect after paclitaxel infusion correlates with the rare occurrence of PPE with this drug. Whether there is a concentration-dependent or a time-dependent development of PPE lesions after a critical antioxidant decline due to chemotherapeutic treatment remains to be investigated.

The purpose of the present study was to identify a possible mechanism of PPE induction by cytotoxic drugs reported to cause PPE. Since all the chemotherapeutic agents administered in this study showed subsequent antioxidant decay in the skin, it can be assumed that oxidative stress might play a significant role in the pathomechanism of PPE induced by these agents. Therefore, the topical application of an antioxidant-containing ointment could also be effective in the prevention and treatment of PPE associated with

paclitaxel, docetaxel and 5-FU, as was shown for doxorubicin-induced PPE (12, 13).

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