Reparaturwirkungen von Folacin auf Hautschäden durch Strahlentherapie

The repair effect of Folacin on skin damage due to radiotherapy

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Schlüsselwörter
Folsäure (Folacin), Strahlentherapie, Hautschädigung, Hautregeneration

Key Words
folic acid (Folacin), radiotherapy, skin damage, skin regeneration

Zusammenfassung
Obwohl medizinische und pharmakologische Fortschritte unübersehbar sind, ist die Behandlung bösartiger Tumore nach wie vor mit einem hohen Risiko unerwünschter Nebenwirkungen verbunden. Insbesondere bei der Strahlentherapie sind allgemeine Nebenwirkungen (Krankheitsgefühl und Unwohlsein) und lokale Nebenwirkungen wie kutane Strahlenschäden zu beobachten. Kutane Strahlenschäden bedürfen einer entsprechenden Behandlung und Pflege sowohl während der Radiatio als auch nach Beendigung der Strahlentherapie. Viele Patienten greifen dabei nach kosmetischen Produkten, die die vorher geschädigte wieder in eine eine gesunde Haut zurückführen sollen. Ziel dieser Studie war es, Wirksamkeit, Tolerabilität und kosmetische Qualität einer Folacin-haltigen Creme während und nach Strahlentherapie zu untersuchen. In vitro wurden Experimente an primären Fibroblastenkulturen vorgenommen: Der Alkalincomet-Assay wurde verwendet, um die Reparaturwirkungen von Folacin auf Röntgenstrahlen-induzierte DNA-Schaden zu erfassen. Bei 41 Patienten mit Strahlentherapie führten wir in vivo Untersuchungen durch. Über nichtinvasive Verfahren wurden die Parameter Erythrem, Feuchtigkeit der Haut und Talgbildung an den bestrahlten Körperregionen (Wangen, Hals oder Brust) jeweils 2, 4 und 8 Wochen nach Behandlung mit der Creme erfasst. Die Repairrate von DNA-Schäden war nach 15–30 min post radiationem höher bei Folat-behandelten primären Fibroblastenkulturen als bei Kontrollen. Unsere Daten sprechen für eine Folsäure-modulierte Reparatur der DANN mit einer rascheren Verknüpfung der Strangbrüche. Wir stellten eine wirksame Verbesserung der Hautparameter durch Folin-haltige Creme unter Radiotherapie fest. Die Anwendung des Verums verminderte i. Vgl. zu Kontrollen Rötung und Couperose, verbesserte aber auch Hautfeuchte und Sebumgehalt. Die Creme wurde sehr gut durch die Patienten toleriert und ihre kosmetischen Eigenschaften waren überzeugend.

Summary
Although medical and pharmacological methods of treatment improve constantly, the treatment of malignant tumors is still connected with high risk of adverse effects of the treatment. It is mostly related to radiotherapy, which causes general (e.g. discomfort and ill-being) and local side effects – it damages the patient's skin in the area that is directly exposed to radiation. Such damages of the skin require appropriate treatment and nursing care, both during the therapy and after the treatment is finished. Many patients search for cosmetic products that could restore the healthy state of their skin, previously damaged by radiation. The aim of this study was to evaluate efficacy, tolerability and cosmetic features of Folacin-containing cream applied on the skin of patients undergoing and after radiotherapy. In vitro research was performed on primary fibroblast culture: the alkaline comet assay was used for the assessment of repairing effect of Folacin on X-ray-induced DNA damage. The in vivo study have been conducted in the group of 41 patients undergoing and after radiotherapy. Non-invasive measurements of skin parameters (erythema, moisturization and sebum level) were preformed on irradiated body skin surface (cheeks, neck or breast) after 2, 4 and 8 weeks of cosmetic treatment. The rate of repair of DNA damage (up to 15–30 minutes) in X-irradiated primary human fibroblasts was higher in folate-treated cells than in untreated ones. Our data suggest that folic acid modulated DNA repair and the observed effects apparently was due to accelerated rejoining of strand breaks. We found that Folacin-containig cream sufficiently improved skin condition in patients undergoinig and after radiotherapy. Application of Folacin-containig cream in comparison to non-treated area significantly diminished skin redness and couperoses and improved moisturization level and sebum content. The cream was very well tolerated by patients and has a very good cosmetic features.

Introduction

Malignant tumors are among the most dangerous diseases that affect mankind. The number of new cases and deaths caused by tumors increase constantly. The most probable reasons of this situation are civilizational changes, which increase probability of falling ill, and longer life expectancy. Although medical and pharmacological methods of treatment improve constantly, the treatment of malignant tumors is still connected with high risk of adverse effects of the treatment. It is mostly related to radiotherapy, which causes general (e.g. discomfort and ill-being) and local side effects – it damages the patient's skin in the area that is directly exposed to radiation. This is the reason of the patient's discomfort. Such damages of the skin require appropriate treatment and nursing care, both during the therapy and after the treatment is finished. Preventive actions and

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the treatment of postradiation reactions should be started during radiation therapy and should last for many months after the therapy is finished. The period when skin damages will appear and the degree of those damages depend upon the type of radiation used in the treatment, its penetrating power, dose and scheme of dose-fractionation, time of exposure, location and size of the area of the skin exposed to radiation. When higher doses and soften radiation is used (soft radiation has longer wave length and is less penetrating), the degree of the skin damages is higher and the symptoms emerge earlier.

In the first stage of radiation therapy reversible changes appear. Those include erythema, lowered activity of the sebaceous and sweet glands and transient epilation. During the following stage, long-lasting reversible hyperpigmentation emerge. The most intense form of the postradiation reactions are irreversible changes, such as scars. These days such damages are observed infrequently, mainly due to advances in equipment used in radiation therapy and the dosimetry of radiation. Deficiency in skin care may lead to intensification of the postradiation changes and prolong their healing. In extreme situations it may lead to permanent cicatrical lesions, ulceration and even to secondary skin cancer [10]. Preventive actions and the treatment of postradiation reactions should be started during radiation therapy and should last for many months after the therapy is finished. Many patients search for cosmetic products that could restore the healthy state of their skin, previously damaged by radiation. Unfortunately, such cosmetics are not commonly available. The aim of this study was to evaluate efficacy, tolerability and cosmetic features of Folacin-containing cream applied on skin patients undergoing and after radiotherapy. Our previous researches showed that Folacin (folic acid) is crucial in personal care products as a regenerating, protective and anti-photo-aging cosmetic ingredient [4–6]. Folacin is well-known vitamin, which deficiency is associated with a number of human diseases and may have cancerogenic effect. It is also an important factor in DNA metabolism. It has been shown that folate deficiency increases background levels of DNA damage, induces genomic instability and excessive misincorporation of uracil into DNA [7, 1, 8, 11].

We used alkaline comet assay to study repair of X-ray-induced DNA damage in primary human fibroblasts growing in the presence of Folacin. Then we evaluated the efficacy and tolerability of Folacin-containing cream in the group of patients undergoing and after radiotherapy according to the breast cancer and malignant tumours of face and neck.

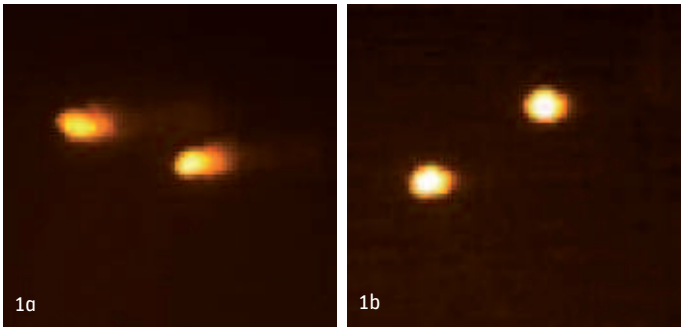


Fig. 1: X-irradiated fibroblasts (a), folate-treated fibroblasts (b). Alkaline comet assay.

Material and Methods

In vitro study

Cell cultures

Primary human skin fibroblasts were grown in standard MEM medium (GIBCO), supplemented with 20 % foetal calf serum (FCS), 10 mM HEPES, 2 mM L-glutamine and antibiotics (100 U/ml penicillin, 0.25 mg/ml streptomycin sulphate) at 37°C in 5 % CO_2 and incubated with 22 mM (0.01 %) folic acid for 10 days or 2 hours prior to radiation. Confluent passages between 2 to 8 were used.

X- irradiation

Exponentially growing cells on 35 mm plastic Petri dishes were irradiated with 3 Gy or 5 Gy of X-rays with an X-ray machine (ANDREX, Holger Andreasen, Denmark, 200 kVp, 5 mA, dose rate 1.2 Gy/min). To determine the initial DNA damage cells were irradiated on ice. For the time course experiments cells were irradiated at room temperature and, after medium change, were incubated at 37°C for the specified period of time.

Alkaline comet assays

We used alkaline comet assay to study repair of X-ray-induced DNA damage in primary human fibroblasts growing in the presence of Folacin. For the alkaline assay the cells were processed as described by Kruszewski et al [9]. Briefly, the cell suspension (4 x 10^5 cells/ml) was mixed with agarose at a final concentration of 1% and cast on microscopic slides as described above. After solidification, the cover slips were removed and the slides placed in the lysing solution (2.5 M NaCl, 100 mM Na_2EDTA, 10 mM Tris, pH 10 and 1% Triton X-100) for 1 h at 4°C. Thereafter, the slides were placed in a horizontal gel electrophoresis unit filled with fresh electrophoretic buffer (1 mM Na_2EDTA and 300 mM NaOH, pH › 13) and left in this buffer for 40 min for DNA unwinding. Without changing the alkali solution the slides were electrophoresed for 30 min at 30 V (1.2 V/cm, 48–53 mA) at 8°C.

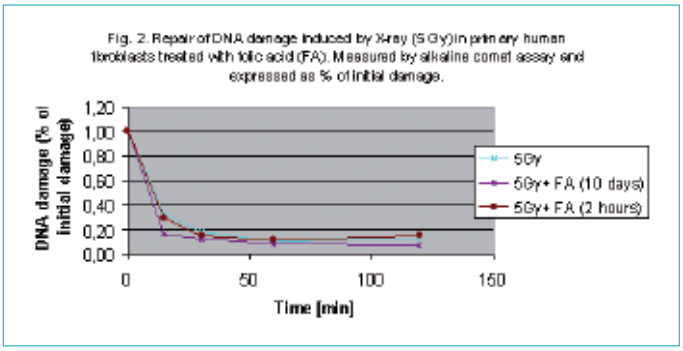
In vivo study

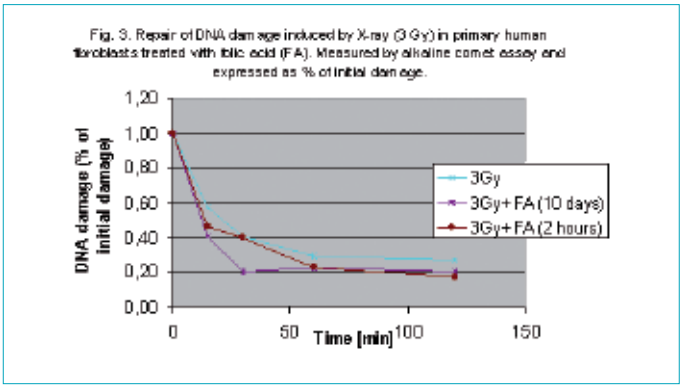
The efficacy and tolerability of Folacin-containing cream were tested in the group of 41 patients (applied twice a day for a period of 8 weeks) undergoing and after radiotherapy according to the breast cancer and malignant tumours of face and neck. Non-invasive measurements of skin parameters (erythrema, moisturization and sebum level) were preformed on irradiated body skin surface (cheeks, neck, breast) after 2, 4 and 8 weeks of cosmetic treatment. Erythrema level was measured using Mexameter SM 815 probe (Courage-Khazaka, GmbH, Niemcy), moisturization level was measured using Corenometer CM 825 probe (Courage-Khazaka, GmbH, Niemcy), content of sebum was measured using Sebumeter 825 probe (Courage-Khazaka, GmbH, Niemcy).

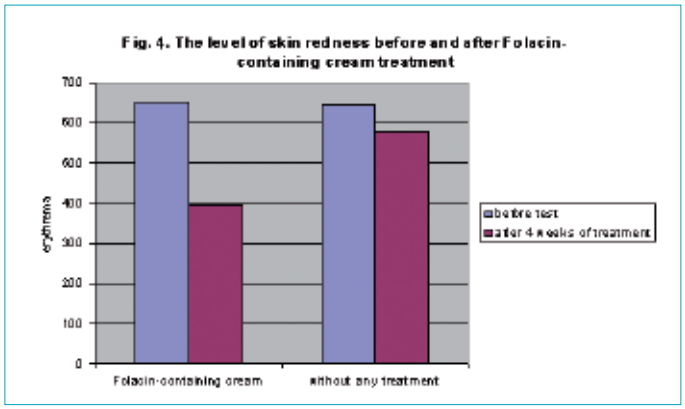
Results

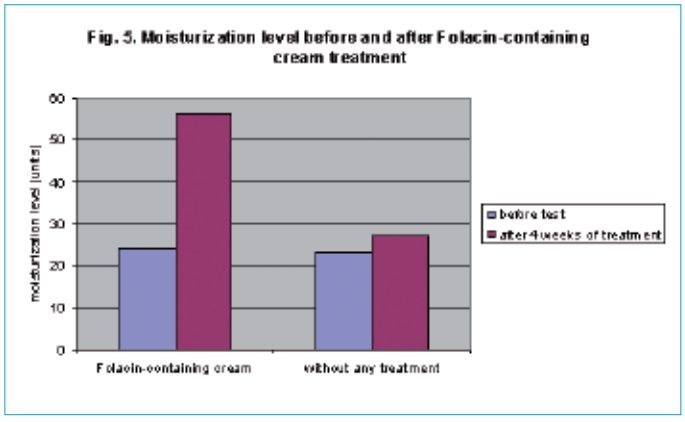
In vitro study

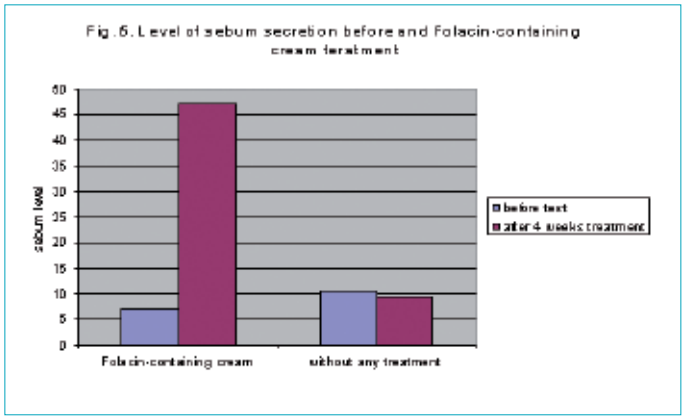
In vitro study showed that the rate of repair of DNA damage (up to 15–30 minutes) in X-irradiated primary human fibroblasts is higher in folate-treated cells than in untreated ones (Fig. 1). We found that cells treatment with Folacin for 10 days increased the rate of repair of X-ray-induced DNA damage:











• after 15 min of repair (5 Gy), DNA damage was reduced to 32.5%, 30.0% and 16.1% of the initial levels in control cells, 2h Folacin-treated cells and in 10 days Folacin-treated cells, respectively (Fig. 2).

• after 30 min of repair (3 Gy), DNA damage was reduced to 40.5%, 39.7% and 20.0% of the initial levels in control cells, 2h Folacin-treated cells and in 10 days Folacin-treated cells, respectively (Fig. 3).

In vivo study

In vivo study showed that the application of Folacin-containig cream significantly improves skin condition. Application of tested cream diminished skin redness and couperoses in the X-ray-treated skin (Fig. 4). We observed an increase of the moisturization level and improvement of sebum content after application of Folacin-containing cream in comparison to non-treated area (Fig. 5–6). The cream was very well tolerated by patients and had a very good cosmetic features (consistency, skin absorption and comfort of use).

Discussion

The radiotherapy of cancer has a very severe influence on exposed skin condition. Repeated exposition on X-radiation causes many different symptoms of skin post radiation syndrome. Depending on used radiation doses the post radiation dermatitis (radiodermatitis) can have acute or chronic course. The main symptoms of acute radiodermatitis are rash, edema, bullous lesions and skin ulceration. The chronic radiodermatitis is a type of poikiloderma, where you can see teleangiectases, hipo- or hyper-pigmentations and skin atrophy. The most common skin problem is skin dryness and sclerosis. The damage of nuclear DNA in skin cells can lead to generation of skin cancers. Such skin problems cause very negative visual effect and influent on patients quality of life. The skin damage due to radiotherapy requires special treatment and skin care regime.

According to the results of our researches we decided to apply the folic acid as a main active ingredient in the cream for patients during and after radiotherapy. Folic acid is involved in the synthesis of S-adenosylmethionine, the primary methyl donor for DNA methylation. S-adenosylmethionine deficiency causes hypomethylation of the deoxyribonucleic acid and undesirable activation of proto-oncogenes. The adequate level of cellular folic acid protects the cells from errors during DNA synthesis and is likely to stimulate repair of existing damage. Our data suggest that folic acid modulates DNA repair and the observed effects apparently are due to accelerated rejoining of strand breaks. We observed that the rate of repair of DNA damage in X-irradiated primary human fibroblasts, depend on dose radiation, is higher in folate-treated cells than in untreated ones. The skin treated with radiation therapy should not be exposed to sunlight, even for many months or years after the treatment. However, in daily

activities it is very difficult to avoid or eliminate the influence of ultraviolet radiation. Our previous in vitro studies showed that DNA damages caused by exposure to ultraviolet are repaired faster in the skin cell that were cultured with folic acid [3, 5, 6]. During an independent study performed on epidermis cells, which are physiologically exposed to the highest doses of ultraviolet, it was showed that folic acid prevented ultraviolet-induced apoptosis (programmed cell death). The level of apoptosis was measured using techniques such as ELISA and flow cytometry, which monitored characteristic morphological changes in apoptotic cells [2, 4]. The results of the studies prove that local availability of folic acid is an additional protection for skin cells against possible damages induced by sunlight.

The results of our study showed that Folacin-containig cream improves skin condition in patients with different stages of skin postradiation syndrome. It was proved that if the cream is used (since the third week of the radiation therapy), it significantly improves the patients' skin condition and their general feeling. It was observed that skin reddening is less intense and the skin is more moisturized and oiled. The cream has very good soothing properties and it improves the toleration of postradiation reactions. It affected mostly patients having radiation therapy due to head and neck tumors, as these tumors require higher doses and therefore making intense postradiation reactions more frequent. Appropriate care of the skin damaged by radiation will improve general well-being of the patients and increase physical and mental comfort of their lives.

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