

The Repair Effect of Folacin Reducing Skin Damage Due to Radiotherapy

**R. Debowska¹, C. Vincent¹, M. Kruszewski²,
B. Winkler-Spytkowska³, A. Maciejczyk³,
K. Rogiewicz¹ and I. Eris¹**

¹Research & Development Department, Dr Irena Eris Centre for Science and Research, Warsaw, Poland, 02-595; ²Department of Radiobiology and Health Protection, Institute of Nuclear Chemistry and Technology, Warsaw, Poland, 03-195 and ³Teleradiotherapy Department, Lower Silesian Oncology Center, Wroclaw, Poland, 53-413.

Introduction

Folic acid (folacin) is an important factor in DNA metabolism. The folacin deficiency is associated with a number of human diseases and may have cancerogenic effect. It has been shown that folate deficiency increases background levels of DNA damage, induces genomic instability and excessive misincorporation of uracil into DNA¹⁻⁴.

The aim of this study was to prove the repairing effect of folic acid (folacin) on X-ray-induced DNA damage in primary human fibroblasts. We also evaluated efficacy, tolerability and cosmetic features of folacin-containing cream applied on skin of the patients undergoing radiotherapy.

Material

Primary human skin fibroblasts were grown in standard MEM medium (Eagle's), (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% CO₂ and incubated with 22 mM (0.01%) folic acid for 13 days or 2 hour prior to radiation. Confluent passages between 2 to 8 were used.

The efficacy and tolerability of folacin were tested in the group of 41 patients undergoing the radiotherapy according to the breast cancer and malignant tumours of face and neck.

Methods

Exponentially growing cells on 35 mm plastic Petri dishes were irradiated with 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.

We used alkaline comet assay to study repair of X-ray-induced DNA damage in primary human fibroblasts growing in the presence of Folicin.

For the alkaline assay the cells were processed as described by Kruszewski et al. ⁵. Briefly, the cell suspension (4×10^5 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 Na₂EDTA, 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 Na₂EDTA 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.

Non-invasive measurements of skin parameters (erythema, moisturization and skin sebum level) were performed on irradiated body skin surface (cheeks, neck or breast) after 2, 4 and 8 weeks of cosmetic treatment. Erythema 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). The tested were used twice a day for a period of 8 weeks.

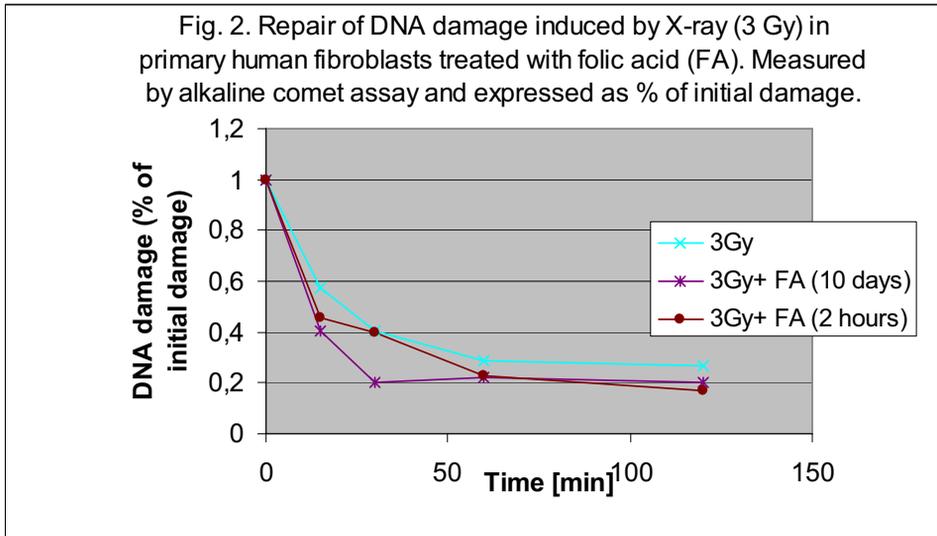
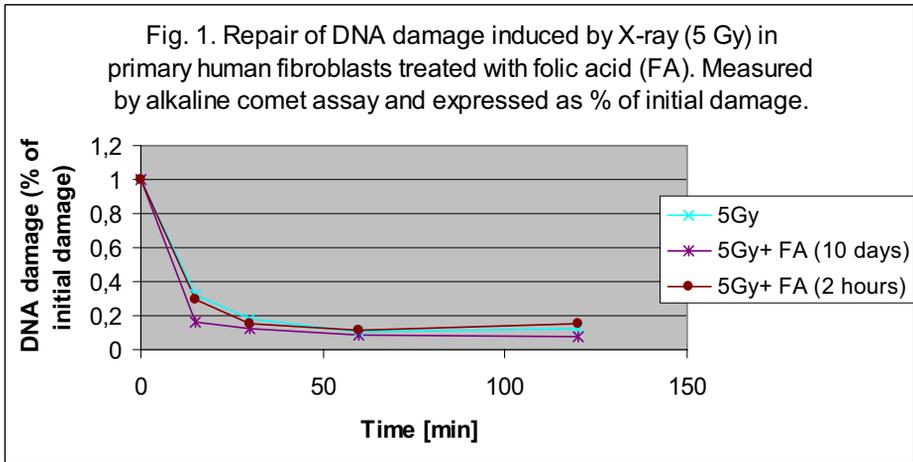
Results

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. We found that cells treatment with Folicin 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 Folicin-treated cells and in 10 days Folicin-treated cells, respectively, Fig. 1
- 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 Folicin-treated cells and in 10 days Folicin-treated cells, respectively, Fig. 2.

In vivo study showed that the application of folicin-containing cream significantly improves skin condition. Application of tested cream diminished skin redness and couperoses in the X-ray-treated skin (Fig. 3). We observed an increasing the level of moisturization and improvement of sebum content after Folicin-containing cream in comparison to non-treated area (Fig. 4-5).

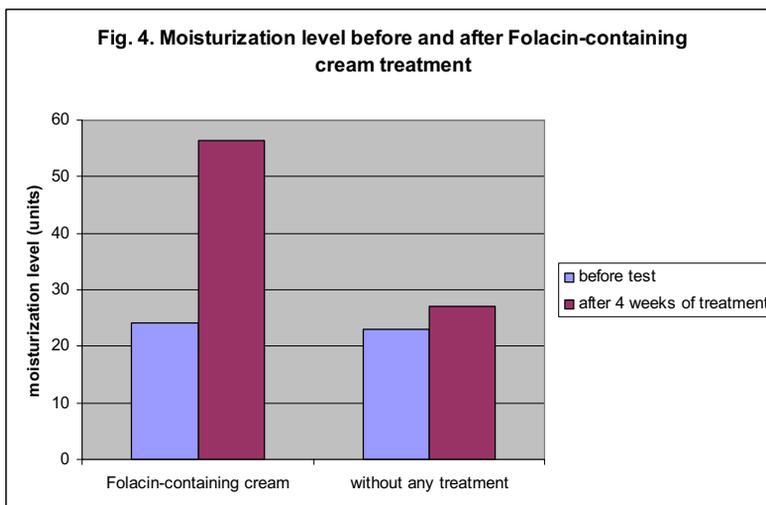
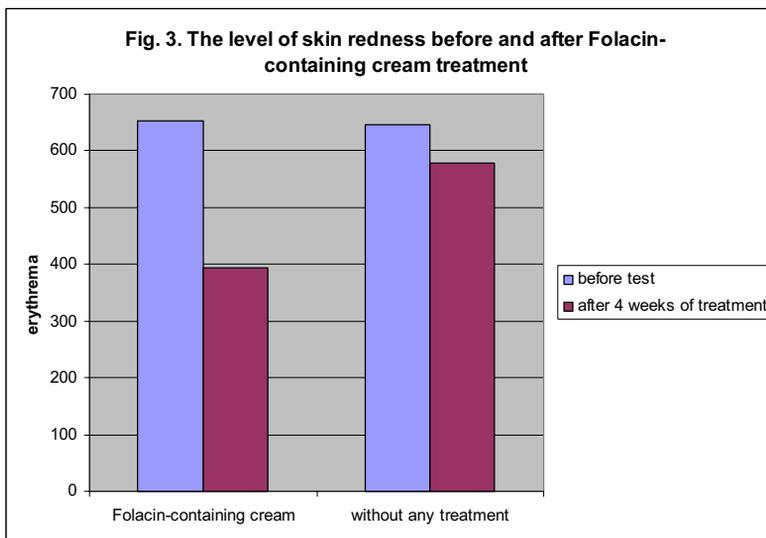
The cream was very well tolerated by patients and had a very good cos-



metic features (consistency, skin absorption and comfort of use).

Discussion

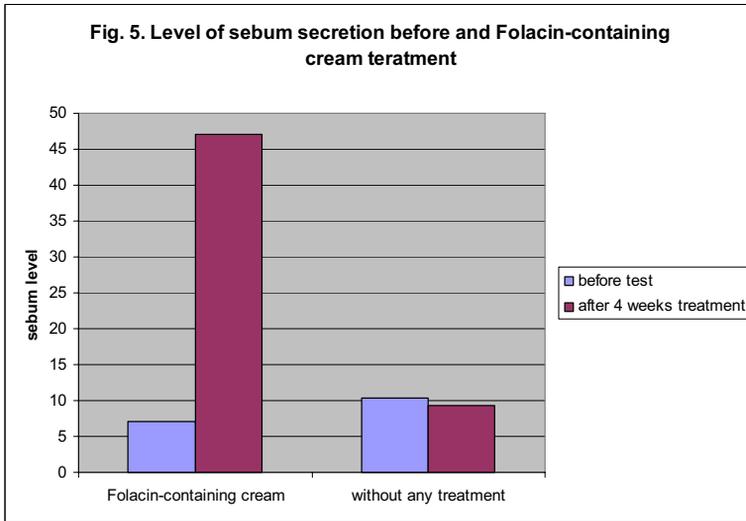
The radiotherapy of cancers has a very severe influence on exposed skin condition. Repeated exposition on X-radiation causes many different symptoms of skin post radiation syndrome. Depending on used radiation doses the post radiation dermatitis (radiodermatitis) can have acute or chronic course. The main symptoms of acute radiodermatitis are rash, edema, bullous lesions and skin ulceration. The chronic radioderatitis is a type of poikiloderma where



you can see teleangiectases, hypo- or hyperpigmentations 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 research we decided to apply the folic acid as a main active ingredient in the cream for patients during and after radio-



therapy. 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.

The results of our research show that folacin-containing cream improves skin condition in patients with different stages of skin post radiation syndrome.

References

1. Kim YI, Pogribny IP, Basnakian AG. Folate deficiency in rats induces DNA strand breaks and hypomethylation within the p53 tumor suppressor gene. *Am J Clin Nutr* 1997 Jan; 65(1): 46-52.
2. Blount BC, Mack MM, Wehr CM. Folate deficiency causes uracil misincorporation into human DNA and chromosome breakage: implications for cancer and neuronal damage. *Proc Natl Acad Sci* 1997; 94: 3290-5.
3. Kruman I et al. Folic Acid Deficiency and homocysteine impair DNA repair in hippocampal neurons and sensitize them to amyloid toxicity in experimental models of Alzheimer's disease. *Journal of Neuroscience* 2002; 22: 1752-1762.
4. Landhaler M., Hagspiel H.J., Braun-Falco O.: Late irradiation damage to the skin caused by soft x-ray radiation therapy of cutaneous tumors. *Arch Dermatol* 131:182-186,1995
5. Ramaekers VT, Reul J, Kusenbach G. Central pontine myelinolysis associated

with acquired folate depletion. *Neuropediatrics* 1997; 28: 126-30.

6. Kruszewski M, Green M H, Lowe J E, Szumiel I. DNA strand breakage, cytotoxicity and mutagenicity of hydrogen peroxide treatment at 4 degrees C and 37 degrees C in L5178Y sublines, *Mutat. Res.* 1994; 308: 233-241.