Folsäure (Folacin) – Neue Neue Anwendungsmöglichkeiten eines kosmetischen Wirkstoffes

Folic Acid (Folacin) – New Application of a Cosmetic Ingredient

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Schlüsselwörter
Folsäure, Vitamine, Anti-Photoageing, Hautregeneration

Key words
folic acid, vitamins, anti-photo-ageing, skin regenerating

Zusammenfassung
Viele Jahre der Versuche und Forschungstests haben bewiesen, dass zahlreiche, wohlbekannte Vitamine in der Kosmetologie erfolgreich verwendet werden können. Die verfügbaren Daten deuten darauf hin, dass eines von denen – Folsäure – eine wichtige Rolle im Lebensprozess der mitotisch aktiven Gewebe spielt und dessen Mangel das Hintergrundniveau der DNA-Beschädigung steigert. Folsäure scheint über Hautregenerationseigenschaften zu verfügen und kann die DNA-Reparatur in der UV-geschädigten Haut modulieren. In der vorliegenden Studie haben wir die Möglichkeit überprüft, Folsäure in Körperpflegeprodukten und Kosmetika als einen Anti-Photoalterungsbestandteil zu verwenden.

Zunächst wurde eine in vitro Untersuchung an der primären Fibroblastenkultur durchgeführt: Die Entwicklungsfähigkeit der Zellen wurde im Burker-Zellen-Gerät bestimmt; die Form der Zellen wurde mit konfokaler Mikroskopie beobachtet; der basische Comet-Test wurde zur Einschätzung der Reparaturaktivität bei UV-induzierter DNA-Schädigung benutzt. Die in vivo Untersuchung wurde an einer Gruppe von 30 Probanden durchgeführt. Wir haben Hautbefeuchtung, Talgsekretion, Elastizität, transepidermaler Wasserverlust (TEWL) und Mikrotopographie des Unterarms und der Hautoberfläche gemessen.

Abhängig von der Konzentration hat die Folsäure die Entwicklungsfähigkeit der primären menschlichen Fibroblasten gesteigert und ihre Proliferation stimuliert. Im Gegensatz zu den Kontrollzellen waren die mit Folacin behandelten Zellen sehr regelmäßig in Form (spindelförmig) und mit guter Fortpflanzungsfähigkeit. Interessanterweise stellten wir fest, dass die Behandlung mit Folsäure die Reparaturrate der UV-induzierten DNA-Schädigung gesteigert hat. Unsere Daten weisen darauf hin, dass Folsäure die DNA-Reparatur moduliert und, dass die beobachteten Effekte offenbar infolge beschleunigter Wiederverknüpfung der Strangbrüche vorkommen.

In vivo Tests haben gezeigt, dass eine 30-tägige Behandlung mit einer Folacin beinhaltenden Créme die Hautbefeuchtung steigern konnte und den TEWL reduzierte, ohne signifikant die Talgsekretion zu ändern. Die Hautelastizität war nahezu zweimal höher nach der Benutzung der getesteten Créme und die Analyse der Mikrotopographie hat reduzierte Hautrauhigkeit, verminderte Anzahl an Hautrissen und Unregelmäßigkeiten sowie einen kleineren Desquamationsindex gezeigt.

Summary
Many years of trials and research tests proved that a lot of well-known vitamins could be successfully used in cosmetology. The available data indicate that one of them – folic acid plays an important role in life process of mitotically active tissues and its deficiency increases background level of DNA damage. Folic acid seems to have skin regeneration properties and it can modulate DNA repair in UV-damaged skin. In this study we check the possibility of using the folic acid (folacin) in personal care products and cosmetics as an anti-photo-aging cosmetic ingredient.

At first in vitro research was performed on primary fibroblast culture: cell viability was determined in a Burker chamber; the shape of the cells was observed using confocal microscopy; the alkaline comet assay was used for assessment repairing activity of UV-induced DNA damage. The in vivo research has been conducted in a group of 30 volunteers. We have measured skin moisturisation, sebum secretion, elasticity, transepidermal water loss and micro topography of the forearm and skin face.

The folic acid, depending on concentration, improved viability of the primary human fibroblasts and stimulates its proliferation. The folacin-treated cells in contrast to the control cells were very regular in shape (spindle-shaped) with high ability of reproduction. Interestingly, we have found that treatment with folic acid increased the rate of repair of UV-induced DNA damage. Our data suggest that folic acid modulates DNA repair and the observed effects apparently are due to accelerated rejoining of strand breaks.

In vivo tests showed that 30-day treatment with cream containing folacin improved the skin moisturisation, decreased TEWL without any significant change of sebum secretion. Skin elasticity was almost two times greater after using tested cream and analysis micro topography showed decrease of skin roughness, number of trough and irregularity and desquamation index.

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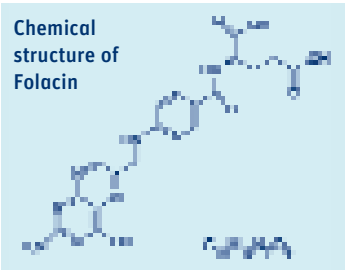
Introduction

Vitamins have been known to play an important role in human life. They must be supplied in the diet or in dietary supplements because they are necessary for our growth, vitality, and general well-being. Many of them, including folic acid, are not produced in human organism. Vitamins help regulate metabolism, help convert fat and carbohydrates into energy, and assist in forming bone and tissue. A lot of people think vitamins can replace food. In fact, it is not really true.

Some of them, like vitamin A and carotenoids, vitamin C and E or their derivatives, vitamin B5 (D-panthenol, D-panthotenic acid) and vitamin F (unsaturated essential fatty acids and their esters) after many years of improvements, trials and research have been used very successfully in cosmetology [8, 17]. Vitamin A and its derivatives have a comprehensive effect on the skin: they facilitate anti-acne treatment, stimulate biosynthesis of proteins and DNA in the epidermis, protect against air pollutants and free radicals, are used in the treatment of furunculosis and other skin lesions [8]. D-panthenol has the ability to attract and retain water. Due to its invaluable physico-chemical properties, D-panthenol can be used as a vehicle for other cosmetic ingredients. It facilitates application and spreading of cosmetic products [7]. Vitamin C regulates biosynthesis of hormones, collagen and neurotransmitters; helps maintain the normal concentration of copper, manganese and zinc ions [4, 7, 16]. It is an antioxidant able to regenerate oxidized forms of vitamin E. While vitamin C is a water-soluble antioxidant, vitamin E is an excellent liposoluble antioxidant. It does not transform into a free radical, thus stopping the chain of free radical reactions. Thanks to its structure, vitamin E has a considerable affinity for cell membranes and thus facilitates a preventive effect on unsaturated fatty acids – oxidation sensitive compounds of the cell membranes [15].

Cosmetic products which contain "new" vitamins are currently available, e.g. vitamin K, used in various circulatory disorders. Its antihemorrhagic effect and the ability to prevent bruising used to be employed in medicine only (administered orally or injected in order to accelerate healing of subcutaneous blood extravasations, which appear as a result of traumas and accidents, as well as surgical and plastic operations). Although many people still share the opinion questioning the efficacy of vitamin K in cosmetic products (it is poorly absorbed through the epidermis and does not penetrate into the internal organs, while only via transformation in the liver the vitamin is turned into its active form), the latest scientific reports, clinical studies, and in the first place the physicians' and patients' observations have unequivocally verified the surprisingly high efficacy of creams containing vitamin K [3, 20].


Chemical structure of Folacin

Another "rejuvenated" vitamin used in cosmetics is folacin (folic acid). This vitamin of B group described as vitamin Bc, B_9 or vitamin M, was isolated many years ago from spinach leaves and its name is derived from its common occurrence in green leaves (folium – leaf). Augier established the chemical structure of folic acid in 1946 as a derivative of pteridine, glutamic and p-aminobenzoic acid (Sch. 1).

Folic acid, water-soluble after protonation of the amino groups, plays a role in all kinds of reversible oxidation-reduction reactions and acts as coenzyme for the many enzymes. It is necessary in the synthesis of erythrocytes and plays a crucial role in quickly dividing cells (digestive tract mucosa, hematopoietic system, fetus tissue).

The epidemiological studies indicate that folic acid alone or in a multivitamin supplement plays a crucial role in many cellular processes. It reduces risk of neural tube defects and led to declines in homocysteine levels (protect against atherosclerosis) [5, 9]. Folic acid deficiency may facilitate several different age-related diseases, including coronary artery disease, cerebral infarction and stroke, colon, oesophageal and cervical cancer [2, 11]. Folic acid is an important factor in DNA metabolism. Many studies have showed that background level of DNA damage is elevated in the condition of folate deficiency. Folate is critical for the synthesis S-adenosylmethionine, so its deficiency might result in DNA damage as well as aberrant patterns of DNA methylation [11]. Alternation in DNA methylation has been observed to change the conformational configuration of DNA and to enhance its instability by making it more susceptible to cleavage by endonucleases. Chromosomal breakage at characteristic fragile sites has long been observed when mammalian cells are grown in folate-deficient media [19]. Folate is an essential for de novo biosynthesis of purines and thymidylate, thereby affecting DNA replication and cell division (folate is required for transferring one carbon units in the de novo synthesis of nucleotides). So the chronic folate deficiency may be carcinogenic, induces genomic instability and excessive misincorporation of uracil into DNA or induces micronucleus formation and extensive chromosome damage [2, 11].

In 1996 FDA issued a regulation requiring enriched cereal and grain products in 140 µg of folic acid per 100 g [9]. This regulation is effective since 1998. Folic acid affects cell division, thus the process of their renewal. The available data indicate that folic acid plays an important role in mitotically active tissues – thus it can have skin cell regenerating properties by supplementing necessary micronutrients. There are thus many possibilities to use it in personal care products and cosmetics. So far folic acid has not been used in cosmetics. Small amounts of this vitamin have been only observed in cosmetic preparations based on algae. Folacin deficiency is quickly observed at those who take sunbaths or solaria due to engagement of the folic acid in the repair of skin damage. UV-A and UV-B radiation cause genetic damage and together with folacin deficiency may cause skin cell death. It seems to be the reason to check if the folic acid improves viability of the UV-damaged skin cells by modulation of DNA-repair mechanism. According to this the folic acid can be used in personal care products as a great regenerating and anti-photo-aging ingredient. The aim of this study was: to investigate the effects of folic acid on skin cell proliferation in vitro, to evaluate the DNA-repair mechanisms after UV-radiation in folacin-treated cells, to check the possibility of using the folic acid in personal care products as an anti-photo-aging cosmetic ingredient.

Materials

Primary human skin fibroblasts NHDF-Ad (Biokom) were grown in standard MEM medium (Eagle's), (GIBCO), supplemented with 20 % fetal calf serum (FCS), (GIBCO), 10 mM HEPES, 2 mM L-glutamine and antibiotics (100 U/ml penicillin, 0,25 µg/ml streptomycin sulfate) at 37° C in 5 % CO_2. Passages between 2 to 8 were used.

Methods

Cells viability

Cells (plated at 10 x 10^4 / ml) grew in the presence (sample) or absence (control) of folic acid (Roche) – FA (concentrations: 0,001 %; 0,005 %; 0,01 %) in medium containing 10 % FCS or without FCS. The cultures were maintained for 21 days, the medium was changed every 7 days. Cell viability and number were determined in a Burker chamber by Trypan blue exclusion [3].

Microscope analysis

For cytoskeleton observation cells were cultured on coverslips coated with 1 % gelatin in medium containing 0,01 % folic acid for two weeks. After 14 days of culture cells fixed in 4 % paraformaldehyde were incubated with Falloidin-TRITC complex (Sigma) or with anti-vimentin monoclonal antibody and with secondary anti-mouse-FITC antibody (Sigma). Cells were analyzed in a confocal microscope (LEICA TSC SP2 Spectral & Confocal Microscope).

UV irradiation

Exponentially growing cells (incubated with 0,01 % folic acid for 13 days or 2 hour prior to radiation) were irradiated on 35 mm plastic Petri dishes. UV-C irradiation (254 nm) was carried out at room temperature at a fluence of 20 J / m^2 using a UV illuminator-FLUO-LINK FLX-20M (Biometra). The excision repair of UV-C induced lesions was allowed to proceed during 5, 15, 30 min at 37° C. Thereafter the cells were trypsinized and suspended in MEM medium and comet slides were prepared as indicated below. UV-B irradiation was carried out using a fluorescent bulb TL12 (Philips) that emits most of its energy within a range of 290–320 nm (emission peak at 313 nm). Confluent cells were exposed (250 J / m^2) through phosphate buffered saline (Sigma). Thereafter the cells were trypsinized and suspended in MEM medium. Viability of fibroblasts was determined by Trypan blue exclusion [3].

Alkaline comet assays

For the alkaline assay the cells were processed as described by Kruszewski et al. [12]. 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 a 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.

Image analysis

Pictures of 75 randomly selected comets per slide, from 2 slides in 3 separate experiments were captured at 200 x magnification using an epifluorescence microscope (Labophot-2, Nikon) equipped with a UV-1A filter block (an excitation filter of 365 / 10 nm and a barrier filter of 435 nm). Image analysis of the data was by the Comet v.3.1 (Kinetic Imaging Ltd., Liverpool, UK). The measure of damage was tail moment (fraction of DNA in the tail times tail length). Data analysis was based on the mean population response or on the distribution of damage among cells. Statistical evaluation and plots were prepared with Statistica 5.1 software (StatSoft, Inc Tulsa, USA).

In vivo tests

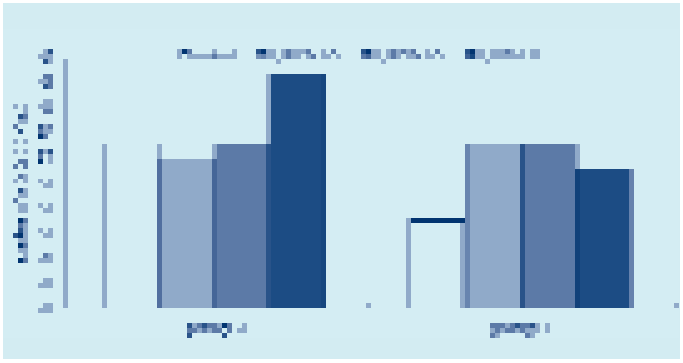


Fig.1: Proliferation of fibroblasts grown for 7 days in the medium containing 10 % FCS with different folic acid (FA) concentrations.

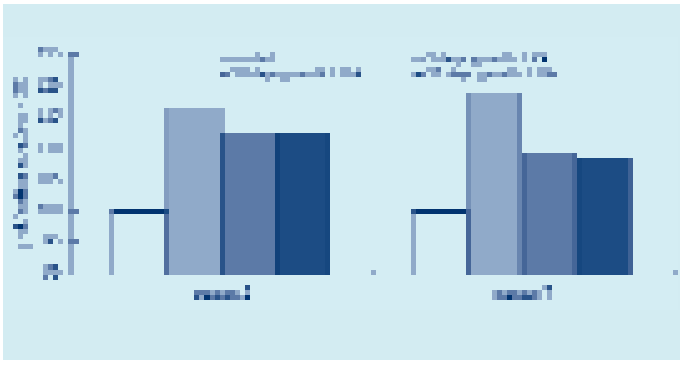


Fig. 2: Proliferation of fibroblasts growing in the medium containig 10 % FCS in the persence of 0,01 % folic acid (FA).

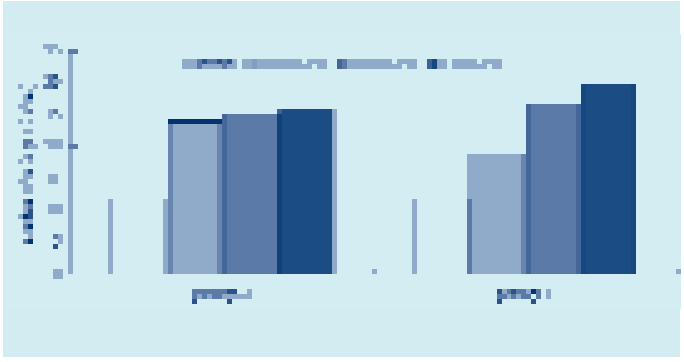


Fig. 3: Proliferation of fibroblasts grown for 7 days in the medium without FCS in the presence of different folic acid (FA) concentrations.

The tests were conducted on 30 volunteers; healthy women aged 26–78 (40,5 ± SD 14,0) with visible ageing symptoms (wrinkle, dry skin), who used folic acid cream every day for 4 weeks of regular treatment. Each volunteer was examined by using several different methods of skin assessment. The examinations were carried out on the indicated places of face and forearm. We measured:

- Skin moisturisation (using Corneometer® CM820)
- Skin greasiness (using Sebumeter® SM 810)
- Skin elasticity (using Cutometer® SEM 575)
- Roughness of the skin surface (using VISIOSCAN® VC98)
- TEWL (using Tewameter® TM 300)

before and after the 2- and 4-weeks of treatment. After the 4-weeks of treatment each volunteer filled in a special questionnaire to assess the efficacy and cosmetic features of the tested product. A preliminary head-to-head comparative study was conducted with a cream containing folic acid versus folic acid-free cream (placebo). In this double-blind study, 30 women between 25 and 60 years of age (mean age 43,4 ± SD 10,8) were using the products 50 / 50. The evaluation was performed in the aforementioned way.

Statistical analysis

The statistical analysis was based on paired t-Student test; p-value < 0,05 was statistically significant [18]. The results are mean value of the skin parameters obtained from participants, "±"standard deviation (SD).

Results

1. Effects of folic acid on fibroblast proliferation

Folic acid favourably affected cells grown in the presence of FCS nutrients (Fig. 1). The number of fibroblasts during 7 days culture in medium containing 0,01 % folic acid increased by about 16 % and 12,5 % in comparison to controls for passage 2 and passage 7, respectively. The ability of fibroblasts in passage 2 growing in the presence of 0,01 % FA to divide was maintained for 21 days of culture, whereas in passage 7 the intensity of cell division was the highest in the first stages of culture (7 days, Fig. 2). Very high stimulation of fibroblasts growing in the presence of folic acid was observed in a culture without of FCS components (Fig. 3). Whereas stimulation of cell proliferation in passage 2 in the presence of various FA concentrations was similar, in the case of cells in passage 7 a higher intensity of cell division was observed with an increase in FA concentration.

2. Microscopy analysis

An observation of cellular morphology was conducted, with folate-treated cells and cells not exposed to folic acid. Actin microfilaments and vimentin intermediate filaments, which form the cytoskeleton, were under scrutiny.

Cytoskeleton observation in non-treated cells

Cells not exposed to folic acid (control) were broad, joined to each other densely by numerous protuberances, were typically asteroid in shape, in most cases a single cell nucleus was visible (Fig. 4 a, 4 c). Mainly non-dividing cells were observed, probably releasing contact inhibitors into the medium, gigantic and mononuclear – probably due to degeneration. The shape indicates stationary phase cells with a large area of cytoplasm – these are probably fibrocytes.

Cytoskeleton observation in folacin-treated cells

Cells exposed to 0,01 % folic acid were numerous, with a small volume of cytoplasm, spindle-shaped, narrow, often lying very close to each other, and flanking each other – probably directly after finishing mitosis. The small size of the cells indicates that they are dividing rapidly (Fig. 4 b, 4 d).

3. Effect of folacin on UV radiation cells

Here, we use alkaline comet assay to study repair of UV-induced DNA damage in primary human fibroblasts growing in the presence of folic acid. To see whether folate influenced the rate of repair of DNA damage, parallel experiments with cells untreated or treated with folate (for 2 hours or 13 days) were performed (Fig. 5). In folacin-treated and than UV-C irradiated cells we observed a rapid increase in DNA damage due to the nucleotide excision repair that generates transient DNA breaks. It was almost 30 % difference after 15 min. of DNA repair between control and folacin-treated cells. Interestingly, in cells treated for 13 days with Folacin, we observed a transient decrease in the number of DNA breaks after 60 minutes of repair (data not presented). This effect had been observed neither in control cells, nor in cells treated with Folacin for 2 hours. Our data suggest that folic acid modulates DNA repair and the observed effects apparently are due to accelerated rejoining of strand breaks.

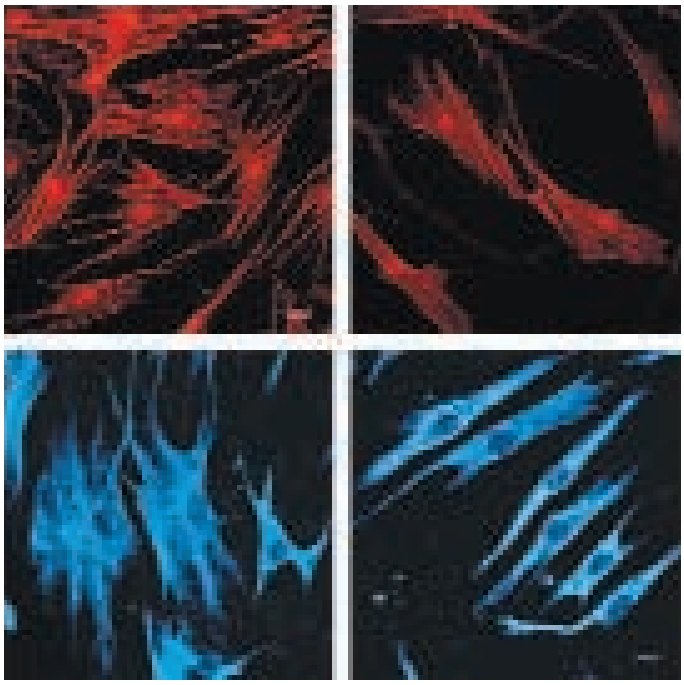


Fig. 4: Fibroblasts cytoskeleton under confocal microscopy observation. Cells was dyeing with Falloidin-TRITC complex (red) or with anti-vimentin monoclonal antibody (green). Control cells were broad, typically asteroid in shape. Folacin-treated cells were narrow, regular and spindle-shaped. A (control) – magnification x 40, B (folacin-treated) – magnification x 54, C (control) – magnification x 66, D (folacin-treated) – magnification x 93.

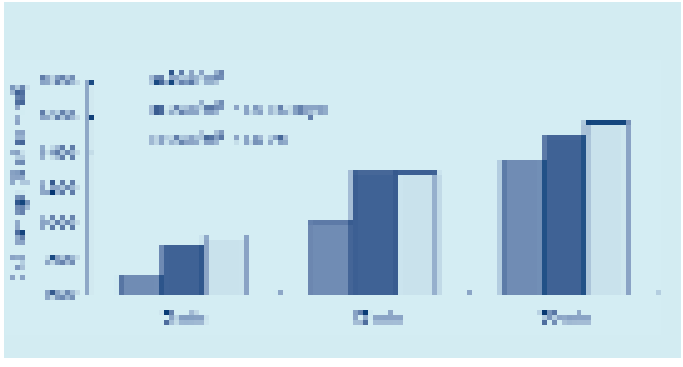


Fig. 5: DNA migration associated with incomplete excision repair sites after exposure of primary human fibroblasts treated with folic acid (FA) to UV-C (20 J / m²). Measured by alkaline comet assay and expressed as % of control.

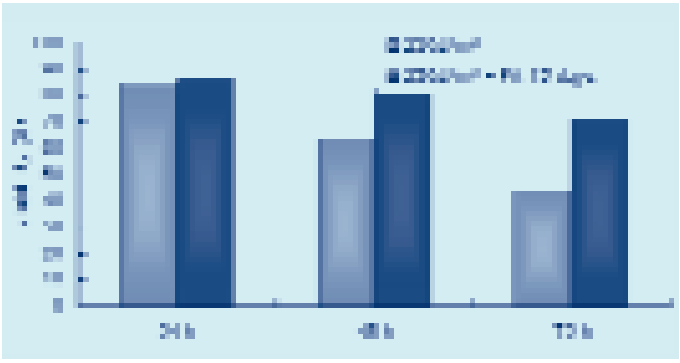


Fig. 6: Viability of the primary human fibroblast culture after UV-B (250 J / m²) radiation. Measured by Trypan blue exlusion.

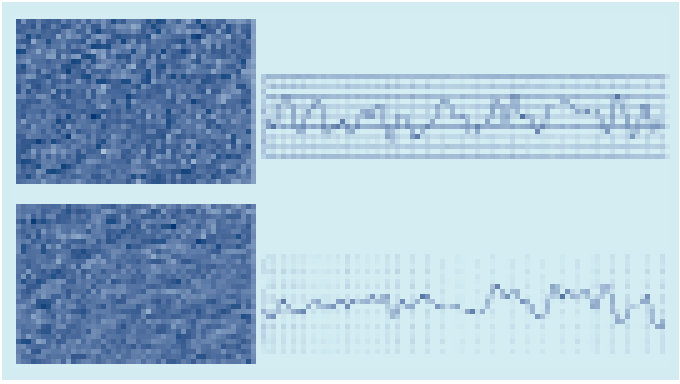


Fig. 7: Skin microtopography and skin profile before and after folacin-cream treatment. Volunteer age 30. Measured by Visioscan VC 98.

We also observed higher viability of folacin-treated fibroblasts than control cells after exposure of UV-B radiation (Fig. 6). The survival rate of cells cultured in the presence of folic acid after exposure to UVB radiation was nearly twice as high as the survival rate of the control cells (not treated with folacin prior to UV exposure).

4. In vivo tests of folacin-containing cream

Moisturisation, secretion of sebum and TEWL

Increased hydration of the skin on the cheeks and forearms was reported after four weeks of regular use of folic acid-containing cream (Table 1). The growth from 84 units to 90 units associated with the skin of the cheeks and from 64,6 units to 76,2 units in the skin of the forearms represented a skin hydration increase of 7 % and 18 % respectively. The lipid content of the skin of the cheeks increased by over 20 % – from 92 µg sebum / cm² to 112,2 µg sebum / cm² of the skin's surface. The increase in lipid content of the skin of the forearms was almost quadruple (Tab. 1). Transepidermal water loss (TEWL) was reduced during the use of products, which contained folic acid. After four weeks of cream application, TEWL reduction from 11,9 g / h / m² to 10,5 g / h / m² was reported in the skin of the cheeks, which represents diminished water evaporation by 12 %. The level of water loss in the skin of the forearms remained unaltered.

Preliminary double-blind placebo-controlled studies (data not shown) revealed no difference in effect on the lipid content of the skin between placebo and folic acid-containing cream. However, there was a statistically significant difference in the skin's hydration level and TEWL in the group using folic acid-enriched cream versus placebo. Although the initial TEWL rates were low – 11,9 g / h / m² (normal healthy skin emits 10–15 g / h / m²) – the TEWL values further decreased (to 10,5 g / h / m²), which suggests reduced permeability of the epidermal barrier [10, 19].

Elasticity

The use of folic acid-containing cream visibly enhanced the elasticity of the skin. The elasticity index of the skin on the cheeks increased by nearly 100 % (from R2 = 0,46 to R2 = 0,79) after 4 weeks of application of the evaluated cream (Tab. 1). The results were statistically significant. Improved elasticity was reported in 80 % of volunteers. Preliminary placebo-controlled studies revealed that the increase in elasticity was the result of use of folic acid-enriched cream.

Analysis of skin surface

Analysis of the surface of the skin on the cheeks was conducted in 18 women prior to and after 4 weeks of use of the indicated cream. The depth, volume and number of creases and skin unevenness diminished in the majority of participants (Volume and Variance index, Table 2). Consequently, the skin waviness index decreased (Surface – measured area of uneven skin surface in relation to flat and perfectly stretched skin). The epidermal roughness and skin scaliness index diminished visibly (by nearly 50 %) (Roughness, Tab. 2). Analysis of microtopography and skin's profile revealed distinct smoothing out of the unevenness of the skin's surface over the zygomatic bone (Fig. 7). Diminished height and width of the peaks in the skin's profile imaging, which represents the height and width of creases and unevenness of the skin, was visible in both 30-year-old and 59-year-old volunteers. The smoothing out of the line, which imaged the skin's profile, was more distinct in 30-year-old volunteers.

Analysis of volunteers' satisfaction

According to the participants' assessment, the product was pleasantly scented, had satisfactory texture and was easy to spread on the skin. The preparation left a delicate protecting film on the surface of the skin. The vast majority of the volunteers felt excellent after the cream application. In subjective opinion of the participants, the skin became smooth, soft and firm.

Discussion

In the present work the effects of folic acid on the growth of human skin fibroblasts in standard medium and in medium depleted of nutrients (no FCS) were analyzed in vitro. A nutrient-poor medium has some analogy to the process of skin aging, as with age the nourishment of skin declines. Folic acid stimulated the division of both young and mature fibroblasts growing in good culture conditions as well as with nutrient deficiencies. It can thus be considered as a factor, which facilitates skin cell regeneration, especially for malnourished cells.

Microscopic observations have confirmed the beneficial effects of folic acid on fibroblast growth and conditions. Actin microfilaments are the finest elements of the cytoskeleton, app. 6 nm in diameter. They are formed as a result of polymerization of actin, a globular protein. These structures are present in all kinds of animal cells, creating a network of tension fibers anchored in the cell membrane by

protein complexes known as attachment plaques. These microfilaments are responsible not only for the movement of organelles and vesicles within the cell but also for exocytosis, endocytosis, formation of filopodia and cell migration [1]. Vimentin intermediate filaments, app. 8–10 nm in diameter, are composed of vimentin protein (54 kD) and are typical for cells of mesenchymal origin (fibroblasts, leukocytes, endothelial cells). Intermediate filaments play crucial role in the maintenance of the three-dimensional cell structure, anchor the cytoskeleton to the cell membrane and support the nucleus and other organelles [1].

Cells cultivated in the presence of folic acid were numerous, small, divided intensely with a small area of cytoplasm. Control cells cultured in standard medium not containing folic acid were large, with abundant cytoplasm, mitotic divisions were sporadically observed. Most of them are probably fibrocytes – stationary non-dividing cells.

Folic acid increases the rate of repair of UV-C induced DNA damage in primary human fibroblasts. Our data suggest that folic acid modulates DNA repair and the observed effects apparently are due to accelerated rejoining of strand breaks. We also observed higher viability of the folacin-treated cells after UV-B radiation than in control cells. The complete mechanism of the effect of folic acid on UV-damaged cells is not known. It is suggested that folic acid affects the synthesis and methylation of the DNA.

The conversion of deoxyuridine monophosphate (dUMP) to thymidine monophosphate (TMP) requires folic acid (5, 10 – methylenetetrahydrofolate), which serves as a methyl donor. Folic acid deficiency, which results in imbalance of deoxyribonucleotide pool, can lead to decelerated and erroneous DNA synthesis. Under conditions of folic acid depletion, a block in the methylation of dUMP to TMP leads to increased cellular level deoxyuridine triphosphate and uracil misincorporation into the DNA molecule (in place of thymine). Enzymes responsible for DNA repair remove the misincorporated uracil. However, if the conversion of dUMP to thymidine is blocked, removed uracil is replaced with another uracil molecule [2, 11]. This causes excessive use of energy required for DNA repair and can lead to more harmful damage – double strand breakage.

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 [11]. Therefore, the adequate level of cellular folic acid protects the cells from errors during DNA synthesis and is likely to stimulate repair of existing damage. The results of in vitro investigations are an indication in the production of effective cosmetic preparations. Essentially each of the „rejuvenated" vitamins has found its place in the cosmetics of the end of the century due to modern analysis of their effects on human skin.

In vivo studies revealed increased hydration of the outer layers of the skin (Tab. 1) as a result of use of folic acid-enriched cream. Retaining proper water gradient in the skin is essential for maintenance of mechanical properties of collagen as well as elastic fibers. Collagen and elastin retain their three-dimensional structure in hydrated form only – water deficiency leads to altered physical qualities of the proteins. Enhanced elasticity of the skin on the cheeks after 4 weeks of use of folic acid-enriched cream (Tab. 1) may result from proper skin hydration and at the same time from normal activity of fibroblasts responsible for collagen synthesis. The presence of folic acid contributes to enhanced division of fibroblasts, increased metabolism, including turnover of collagen and elastin. Mechanical properties of the skin (elasticity) depend on the structure and density of collagen and elastic fibers [14]. Normally functioning skin does not lose the ability to contract and does not get slack, while these factors contribute to the development of wrinkles.

Visibly diminished epidermal roughness (Roughness index, Tab. 2) might have been the result of the thinning epidermal horny layer and increased cell proliferation, enhanced synthesis of natural moisturizing factors (NMF), which distinctly reduce the TEWL [18]. Transepidermal water loss is affected by thickness of the protecting lipid layer of the epidermis. The studies reported that the use of folic acid-containing cream stimulates the synthesis of the protecting barrier, which reduces water evaporation and protects the skin from water loss (which is essential in case of age-related skin dryness). However, the same lipid content-enhancing effect was reported in case of both folic acid-containing cream and placebo (preliminary study,

Features	Before tests	Day 15	Day 30	Statistics significancy
Skin moisturization, units (cheek)	84,1 ±8,6	91,4 ±9,5	90,1 ±7,5	p=0,003
Skin moisturization, units (forearm)	64,6 ±10,4	77,3 ±8,2	76,2 ±9,7	p<0,001
Skin greasiness (cheek) (ug sebum/cm^2)	92 ±57,3	94,4 ±47,0	111,2 ±59,5	p=0,034
Skin greasiness (forearm) (ug sebum/cm^2)	4,5 ±7,5	16 ±17,9	17,6 ±18,0	p<0,001
TEWL (cheek) (g/h/m^2)	11,9 ±3,7	12,7 ±4,1	10,5 ±3,3	p=0,101
TEWL (forearm) (g/h/m^2)	8,3 ±2,0	8,0 ±1,6	7,5 ±2,1	p=0,288
Skin elasticity R2 (cheek)	0,46 ±0,11	0,66 ±0,11	0,79 ±0,16	p<0,001

± SD, T-Student

R2 – portion between the max. amplitude and the ability of redeformation of the skin (gross elasticity). The closer the value is to 1 the more elastic the curve.

Table 1: Improvement of skin condition.

Features	Before tests	After 4 weeks	Number of the with skin improvement*	Statistics people significancy
VOLUME	27,1 ±5,8	19,5 ±2,4	14	p=0,011
VARIANCE	1,92 ±0,23	1,55 ±0,12	15	p<0,001
SURFACE	2,85 ±0,3	2,59 ±0,16	14	p=0,001
ROUGHNESS	0,21 ±0,2	0,1 ±0,14	13	p=0,067

* – the tests were conducted on 18 volunteers

± SD, T-Student

Volume – it calculates the amount of liquid needed in the calculation area to fill the image until the average height of all mountains. The smoother area before filling up, the less liquid is needed. The result is shown in V/mm^2 (one dimensional in gray values).

Variance – it is the average of a local variance over an amount of pixels. The actual value of the pixel is compared with the average.

Surface – this graph shows the size of the „wavy" surface in comparison with the stretched „ironed" surface. The smoother the area was before stretching, the closer the two values are together. The result is displayed in %.

Roughness – calculates the portion of dark pixels corresponding to the wrinkles. It calculates the gray level above the threshold in comparison to the whole image. The smaller value the skin is smoother.

Table 2: Reduction of skin roughness.

data not shown). TEWL and hydration level of the skin significantly improved after use of folic acid-containing cream versus placebo.

Thanks to the local bioavailability of folic acid the cells are able to proliferate intensively, effectively repair damage and synthesize substances essential for normal function of the skin. People who take sunbath or solaria have such a high demand for folic acid that any deficiency is quickly observed. Combined with significant water loss their skin has first skin-ageing symptoms. Sun products should contain folic acid together with UV filters.

Appropriately selected vehicle (liposomes), which carries the active substances, is a crucial element of cosmetic products. Relatively high efficacy of the evaluated cream and absence of any allergic reactions suggest that the vehicle is suitable for sensitive skin as well. Folic acid-enriched cream was well tolerated by the volunteers, did not induce irritations and brought quick, visible and satisfactory results. Subjective assessment of the evaluated product is extremely important, since an effective cosmetic preparation, as opposed to medication, should please, relax and soothe the user. Vitamins currently used in cosmetic products often have completely different effects than ingredients of vitamin-enriched preparations used in the past. This is the result of huge progress in the field of cosmetic technologies and more effective cooperation between cosmetology and medicine, dermatology in particular.

Concluding summary

The folic acid (folacin) improved viability of the primary human fibroblasts and stimulates its proliferation. Treatment with folic acid increased the rate of repair of UV-induced DNA damage. Our data suggest that folic acid modulates DNA repair and the observed effects apparently are due to accelerated rejoining of strand breaks. In vivo tests showed that 30 days treatment of cream containing folacin improved the skin moisturisation; decreased TEWL without any significant change of sebum secretion. Skin elasticity was almost two times greater after using tested cream and analysis microtopography showed decrease of skin roughness, number of trough and irregularity and desquamation index. Folic acid seems to be crucial for normal function of the skin and, especially for UV-exposed skin.

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