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Serum levels of basic Fibroblast Growth Factor in patients with chronic plaque psoriasis

Psoriasis vulgaris is a common chronic inflammatory skin disease affecting approximately 2% of the total population. It is considered to be a T-cell-mediated disease with active hyperproliferation of keratinocytes in the epidermis, increased angiogenesis and cellular infiltration in the dermis. The exact pathogenesis of psoriasis is still unclear, but along with genetic and environmental factors, an immune-mediated process involving many mediators has been implied. Neovascularization appears to play an important part in the evolution of psoriasis. The changes occur early in the formation of psoriatic lesions, preceding any other histological features. Microvessels in the papillary dermis of psoriatic plaques are tortuous and dilated, with increased endothelial surface areas and endothelial cell proliferation (4). These findings indicate an important pathogenetic role of microvascular expansion in lesional skin and have led to the suggestion that psoriasis is an angiogenesis-dependent disease. Several molecules have been reported to affect angiogenesis, many of them are polypeptide growth factors, amongst which basic fibroblast growth factor (bFGF) and vascular endothelial growth factor (VEGF) are the best characterized (3, 8).

bFGF also known as fibroblast growth factor-2 (FGF-2) is one of the 23 members of the FGF family known to modulate angiogenesis, cell growth, differentiation, and chemotactic activity. bFGF is one of the most studied members of the family; it was first identified as 146-amino acid protein isolated from the pituitary. Two different molecular weight bFGF isoforms play an important role in the organism, i.e. the 18-kDa form, which promotes cell migration and mitogenesis, whereas high molecular weight (HMW) FGF controls cell growth. bFGF is expressed by many tissues and has been localized in the basement membrane of blood vessels, muscle, nerve cells, and sweat glands. The cellular source of bFGF is a matter of controversy (1). Many investigators believe that major cellular sources of tissue bFGF are macrophages (1, 15). Some authors point to the mast cells as the possible source of this peptide (13).

Although bFGF is synthesized by many cell types its secretion remains unclear, especially because it lacks a classic leader sequence for secretion (2). It has been suggested that intracellular FGF may be released from cells through passive process in response to cell damage associated with tissue injury or tumor necrosis (12). The released bFGF stays bound to the extra cellular matrix and its controlled release plays a vital role in regulation of angiogenesis.

Outside the cell bFGF is quickly bound to heparan sulfate proteoglycans (HSPGs), which act as coreceptors for FGF-FGF receptor (FGRR) interactions and FGFR signalling (5). HSPGs are associated with most cell surface receptors in the extracellular matrix. The HSPG–bFGF complex serves also as a reservoir for bFGF.

The biologic activity of bFGF is mediated by four related high-affinity transmembrane tyrosine kinase-domain receptors: FGFR-1, -2, -3, and -4, and perhaps other transmembrane receptors (10). On the endothelium FGF interact with FGFR1, the main receptor expressed in endothelial cells. FGF can also interact with FGFR2, which is expressed in small amounts in endothelial cells. It has been reported that FGFR1 stimulation triggers proliferation, migration, protease production and tubular morphogenesis, whereas FGFR2 mediates cell motility. FGFR binding results in receptor dimerization and autophosphorylation. Tyrosine kinase then contributes to endothelial cell proliferation. After entering the nucleus, bFGF is also able to promote endothelial cell proliferation through other pathways. FGF-2 is associated with phosphorylation of nucleolin, resulting in increased transcription of DNA.

bFGF induces endothelial cell proliferation, migration, and angiogenesis *in vitro*, it regulates the expression of several molecules thought to mediate critical steps during angiogenesis. Angiogenesis involves both growth and involution of blood vessels. bFGF seems to promote angiogenesis not only by stimulating the growth of new blood vessels, but also by weakening their apoptotic potential. In addition to activating signalling pathways that result in endothelial cell activation, proliferation, increased survival, migration, and differentiation, bFGF also activates other cell lines, such as smooth muscle cells and fibroblasts, which participate in arteriogenesis. bFGF may be involved in the recruitment or homing of bone-marrow-derived cells into expanding neoarteries. In this way, it is possible that bFGF induces the growth not only of capillaries, but also of more mature arterioles. Moreover, variable local expression of the receptors for FGF receptors may modulate the action of paracrine FGF-2 in arteriogenesis.

The aim of this study was to investigate possible changes in the serum level of bFGF in patients with psoriasis, compare them with normal individuals and correlate them with disease activity.

MATERIAL AND METHODS

A group of 36 patients was involved in the study; 15 females and 21 males (mean age 39 years; age range, 18–65 years). The patients suffered from generalized chronic plaque psoriasis (PASI score 24.83 ± 4.33). The control group consisted of 20 healthy subjects, who took part in the study (mean age 41 years). The patients were evaluated in the outpatient clinic, and the extent and change of the skin lesions were quantified using the Psoriasis Area and Severity Index (PASI) score at study entry, and 3 weeks after treatment. Blood samples were collected from the patients before initiation of treatment, and on the visit in the outpatient clinic 3 weeks after treatment. In addition, blood samples were collected from age- and gender-matched healthy volunteers. Subsequently, the samples were centrifuged and the plasma was carefully separated from the cells and frozen at –80°C until analysis.

bFGF level in the serum was assayed by commercially available enzyme-linked immunosorbent assay kit (R&D Systems, Minneapolis, USA) according to the instruction included in the assays. Each sample was tested in duplicate. bFGF levels were estimated in serum in the active phase of the disease, before the treatment was administered and after clearing of psoriatic lesions, 3 weeks after treatment. The levels of the serm bFGF were estimated within limits 0 - 640 pg/ml. The minimum detectable dose of bFGF was less than 3 pg/ml. The absorbance was read at 450 nm, with the correction wavelength set at 540 nm.

The obtained data were subjected to statistical analysis using the Student's t-test and computer program Statistica. p=0,05 or smaller was considered the relevancy level.

RESULTS AND DISCUSSION

The results are presented in Table 1 and illustrated in Figure 1. Before the treatment was initiated median serum bFGF level was significantly higher among the patients compared with the matched volunteers ($p< 0.001$). After three weeks treatment median serum bFGF decreased significantly in the patients group, still however staying significantly higher than in control group ($p<0.001$).

Table 1. Serum concentration of bFGF (pg/ml) in psoriatic patients in acute clinical stage, before treatment, and in remission period, 3 weeks after treatment

Groups	N	Mean bFGF level (pg/ml) ± SD	Min.	Max
Patients – before treatment	36	7.116 ± 6.65* [a]	1.488	31.291
Patients – after treatment	36	4.229 ± 3.64*	0.210	14.751
Control	20	0.411 ± 0.79	0	2.58

*$p< 0.001$ vs control group, [a] $p< 0.001$ vs patients group in remission (after treatment)

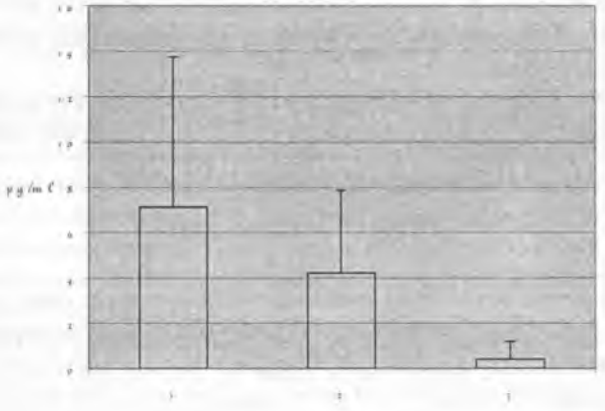

Fig. 1. Serum concentration of bFGF (pg/ml) in psoriatic patients in acute clinical stage, before treatment, and in remission period, 3 weeks after treatment; 1) patients before treatment; 2) patients after treatment; 3) control group

The mean values of bFGF concentration, in the group of patients with psoriasis before the treatment, in the active phase of the illness, were considerably higher compared to the values after the treatment, in the remission phase ($p<0.001$).

The activity of bFGF was an object of study for scientists in the recent years. Deng and co-workers (6) have examined the influence of photochemotherapy (PUVA), used in the treatment of psoriasis, on the bFGF. They have shown in their study that PUVA efficiently inhibited the expression of bFGF in keratinocytes and reduced the total tube length generated by bFGF. The authors postulate that a possible mechanism of photochemotherapy in the treatment of psoriasis may be the inhibition of angiogenesis and induction of apoptosis of human microvascular endothelial cells. Their findings indicate a direct effect of PUVA on endothelial cell migration.

bFGF in neoplasia was also a subject of examination. Recent findings have shown overexpression of bFGF and its receptor in patients with cancer, suggesting that bFGF promotes tumor vascularization and subsequent growth. Elevated levels of bFGF in the serum and urine have been detected in patients with a wide spectrum of cancer and several studies have shown the value of these laboratory parameters as prognostic indicators. Fujimoto and co-workers (9) measured serum levels of bFGF in 31 patients with renal carcinoma and 52% of them showed elevated levels of serum bFGF. The serum bFGF level was also elevated in most stages of breast cancer. Dirix and co-workers showed that 40% patients with untreated metastatic tumors had an elevated level of bFGF (7). The source of circulating bFGF in neoplasia was the subject of disscusion. bFGF is present in the basement membrane, the extracellular matrix, and also in the cytoplasm of tumor cells. Physiologically, the binding of FGF to heparan sulfate in the process of matrix degradation by heparynase leads to the release of bFGF in an active form and subsequently to the growth of capillary blood vessels. It has been postulated that bFGF may be activated by the action of heparine-sulfate-degrading enzymes introduced during tumor development, thus mediating tumor growth and metastasis. It is likely that bFGF is secreted at least partially by tumor tissues in amounts that are sufficient to generate abnormally high levels in serum.

Many present studies concentrate on reciprocal relations between the angiogenic factors, mainly between bFGF, VEGF and TGF-β. Mandriota and co-workers (11) have shown, that bFGF is capable of modulating VEGF-induced angiogenesis *in vitro*. Rifkin and co-workers investigating the opposing effects of transforming growth factor β (TGF-β) on bFGF activity in cultured bovine aortic endothelial cells (14). TGF-β inhibits FGF-2-induced cell migration and protease production. FGF-2 stimulates urokinase type plasminogen activator (uPA) expression, which, in turn, activates latent TGF-β. Activated TGF-β stimulates plasminogen activator inhibitor (PAI-1) synthesis, which inhibits uPA, shutting down subsequent TGF-β formation. This creates a loop regulating both TGF-β activation and FGF-2 activity (3). These data have contributed to the notions that endothelial cell activation status is determined by a balance between positive and negative regulators, rather than by a single regulator alone, and that the angiogenic activity of bFGF depends on interactions with other factors present in the endothelial pericellular environment.

The results of our study confirm the claim that the psoriasis is an angiogenesis-dependent disease, and bFGF is an active participant of pathogenic events in psoriasis. Similarly to many other illnesses, in which angiogenesis is a key element of pathogenesis, for instance neoplasia, in the active phase of psoriasis the bFGF levels are particularly high and bFGF levels correlate with outcome. Assessing the bFGF level may provide prognostic information. Our results suggest that antiangiogenic therapy could be considered a very promising therapeutic method in the treatment of the patients with chronic plaque psoriasis.

CONCLUSIONS

1. In the active stage of psoriasis highly elevated expression of bFGF in serum of 36 patients was found out.
2. The presence of elevated levels of bFGF in peripheral blood and its changes due to treatment indicate that this protein is an active participant of pathogenesis in psoriasis.
3. Measurement of the serum bFGF level may be useful as prognostic information.

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SUMMARY

Changes in the skin microcirculation play an important role in the evolution of psoriasis. The aim of the study was to investigate the activity of the serum level of bFGF, one of the main proangiogenic factors, in the patients with psoriasis. The study was conducted on 36 patients suffering from generalized chronic plaque psoriasis. The mean values of bFGF concentration were assayed by ELISA method in the active stage of psoriasis and 3 weeks after the treatment. Before treatment serum levels of bFGF were significantly raised in patients with psoriasis as compared with control subjects. Clinical clearing was associated with a significant decrease in serum bFGF level, however it still remained significantly higher than in control group. The mean values of bFGF levels in the patients with active stage of psoriasis were considerably elevated compared to the values in the remission period. Our results showed that bFGF is an active participant in pathogenesis of psoriasis.

Stężenia zasadowego czynnika wzrostu fibroblastów w surowicy krwi chorych na łuszczycę

Istotne znaczenie w rozwoju zmian łuszczycowych odgrywają zmiany w mikrokrążeniu. Celem pracy było zbadanie aktywności jednego z podstawowych czynników proangiogennych, zasadowego czynnika wzrostu fibroblastów (bFGF), w surowicy krwi chorych na łuszczycę. Badania przeprowadzono w grupie 36 pacjentów, poziomy bFGF oznaczano metodą ELISA w ostrej, wysiewnej fazie choroby i w okresie remisji, trzy tygodnie po leczeniu. Stwierdzono znacznie podwyższone stężenia bFGF w okresie przed leczeniem u pacjentów łuszczycowych w porównaniu z grupą kontrolną. W okresie remisji obserwowano spadek zawartości bFGF w surowicy pacjentów, jednakże stężenia te pozostawały nadal istotnie wyższe w stosunku do kontroli. Wartości stężeń bFGF w grupie chorych w aktywnym okresie choroby były istotnie statystycznie wyższe w porównaniu z wartością w okresie remisji. Uzyskane wyniki potwierdzają istotne znaczenie bFGF w etiopatogenezie łuszczycy.