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Peroxisome Proliferator Receptor (PPAR) β/δ in psoriatic patients before and after two conventional therapeutic modalities: methotrexate and PUVA

Peroxisome proliferator-activated receptor β/δ is a member of the nuclear hormone receptor superfamily suggested to contribute to psoriasis pathogenesis. Methotrexate and PUVA mainly target the T cell-mediated immunopathology of psoriasis. Our work aimed at estimating PPARβ/δ in psoriatic patients and investigating whether the standard therapeutic modalities (methotrexate and PUVA) exert their anti-psoriatic activity partially through altering PPARβ/δ levels. RT-PCR was used to measure PPAR β/δ mRNA levels in twenty four chronic plaque psoriasis patients. Patients were divided into two groups (12 patients each); group A received intramuscular methotrexate and group B was treated by PUVA 3 times/week in a PUVA 1000 cabin for ten weeks each, followed by measurement of PPAR β/δ mRNA levels. Twelve healthy volunteers served as controls. PPAR β/δ mRNA levels were significantly elevated in all patients and significantly decreased ten weeks after treatment, however, post treatment levels were still significantly elevated in comparison with those of controls. PPAR β/δ mRNA levels showed a significant positive correlation with disease duration.

Key words: methotrexate, peroxisome proliferator-activated receptor β/δ, peroxisome proliferator-activated receptors, psoriasis, PUVA, RT-PCR

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Psoriasis, one of the most prevalent skin diseases, affecting 2-3% of Caucasian populations, is considered to be a polygenetically influenced, immune-mediated, organ-specific disease of dysregulated inflammation that is triggered by environmental factors [1]. Hallmarks of psoriasis are keratinocyte proliferation and altered differentiation as well as T cell activation [2].

Peroxisome proliferator-activated receptors (PPARs) are members of the nuclear hormone receptor superfamily and are expressed in a variety of tissues including skin and cells of the immune system [3]. Several lines of evidence suggest that PPAR beta/delta (PPARβ/δ), known to regulate epithelial differentiation and wound healing, contribute to the pathogenesis of psoriasis [4]. They are thought to act as connectors between the enzymatic mechanisms of the epidermal barrier and the abnormal immune and inflammatory responses that characterize psoriasis [5], as they were found to enhance proliferation and block apoptosis of T cells which contribute to the persistence of activated T cells in psoriatic skin lesions [6].

A wide range of systemic drugs has been developed in recent years for the treatment of psoriasis. Methotrexate (MTX) is one of the classical agents and is still one of the most frequently used systemic treatments for psoriasis worldwide. The mechanism of action is not fully understood, but MTX is suggested to act primarily as an anti-inflammatory and immunosuppressant drug [1]. Psoralen plus UVA (PUVA) phototherapy is also a current mainstay of the treatment of psoriasis, whose target is directly the T cell-mediated immunopathology of psoriasis [7]. Thus it seemed prudent to conduct this randomized, controlled study aimed at estimating the PPARβ/δ in patients with psoriasis and investigating whether the standard therapeutic modalities (methotrexate and PUVA) exert their anti-psoriatic activity partially through altering PPARβ/δ levels.

Patients and methods

Patients

Twenty four patients with chronic plaque psoriasis (18 males (75.0%) and 6 females (25.0%), aged 15-60 years (mean 38.13±13.826 years) with a disease duration ranging from 1-20 years (mean 6.71±5.583 years)) and twelve healthy volunteers (13 males (54.2%) and 11 females (45.8%), aged 16-50 years (mean 27.04±9.082)) with no history of skin or autoimmune diseases who served as controls were recruited from the dermatology outpatient clinic, Kasr El-Aini hospital after approval by the Dermatology Research Ethical Committee (DermaREC) of Faculty of Medicine, Cairo University.

Inclusion criteria included psoriatic patients with an extent of psoriasis of more than 30% body surface area, justifying

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treatment with either methotrexate or PUVA. A pretreatment work up was performed for all patients before being included in the study *(figure 1)*. Exclusion criteria included patients with liver or kidney diseases, uncontrolled diabetes, severe anemia or bone marrow suppression. Also children below 12 years, pregnant and lactating females as well as patients with pustular, erythrodermic or arthropathic psoriasis were all excluded. Any phototherapy, systemic or topical immunomodulatory therapy for psoriasis was stopped at least 8 weeks before being included in the study.

The twenty four psoriatic patients enrolled in our study were allocated randomly to one of two groups, each of which comprised twelve patients according to the treatment modality. Randomization was done using a computer generated random sequence prepared by the statistician and the sequence was kept in the pharmacy. After enrollment, each participant was put in the sequence to determine to which group he/she would be assigned. The randomization key was decoded only after finishing the statistical process. Group A received intramuscular methotrexate in a dose of 2.5 mg/kg/week for ten weeks. Folic acid in an oral dose of 5 mg/day was given to patients except on the day of the methotrexate injection. Group B received PUVA therapy 3 times/week for ten weeks in a PUVA 1000 (Waldmann, GmbH Germany) cabin equipped with an integrated UV radiometer equipped with F85/100-W fluorescent lamps that emits UV light in the wavelength range of 315-400 nm with a peak emission at 355 nm. Patients received 0.7 mg/kg 8-methoxypsoralen (MOP) 2 hours before phototherapy. The UVA dose was started with the minimal erythema dose of 2 J/cm^2 for skin types IV and V and 1 J/cm^2 for skin type III and the dose increased gradually by 0.5 J/cm^2 every other session, according to the patient's response.

The PASI score was documented before treatment, every two weeks and at the final follow-up. Laboratory investigations were done weekly for the first month, then every two weeks. A 4-mm punch skin biopsy was obtained from a particular psoriatic plaque (lesional skin) before initiation of therapy and at the end of the ten weeks from an adjacent area to the previous one from all patients. Biopsies were stored at -70°C and were estimated semi-quantitatively for the level of PPAR beta/delta mRNA using PCR technique. Similar skin biopsies were obtained from the twelve controls.

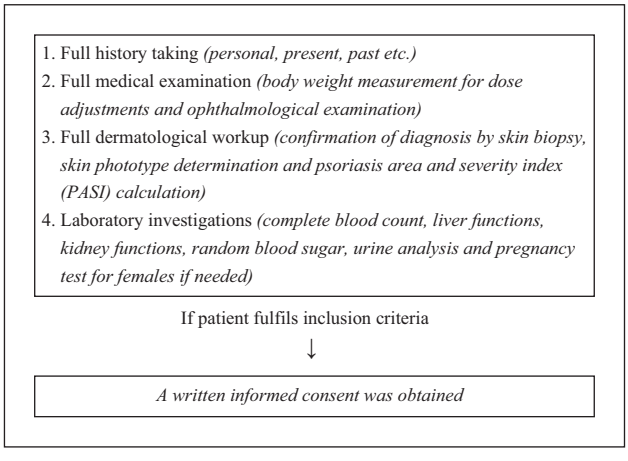

Figure 1. Pretreatment work up for all patients.

Quantitation of the PCR product

RNA was extracted from tissues by using the RNA extraction kit. Reverse transcriptase-polymerase chain reaction analysis of PPAR beta/delta was carried out on extracted RNA using specific primers. The primers sequences are: sense 5-TCCCTCTTTCTCAGTTCCTC-3, and antisense 5-CAGGAGACAGAAGTGAGGAC-3.

Reverse transcriptase-polymerase chain reaction (RT-PCR) was carried out using 5 ug of total RNA extracted from skin biopsies. After denaturation for 10 min at 70°C, RNA was reverse transcribed into cDNA using SuperScript II RNase H– reverse transcriptase (5 units per reaction; Invitrogen, Groningen, the Netherlands). The cDNA was subjected to PCR amplification with the following condition: 5 min. denaturation at 94°C, followed by 30 cycles of: denaturation at 94°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 30 s with final extension for 7 min at 72°C. The PCR mixture contained 1X buffer, oligonucleotides primers, dNTPs, 1 unit of Taq DNA polymerase and cDNA. In every case, PCR negative control (without DNA) was included. The PCR products were electrophoresed on 2% agarose gels stained with ethidium bromide. Positive bands appear at 287 bp.

PCR products were then quantitated by using a quantitation kit (from Promega Corporation, Madison, WI, USA). This method depends on purification of the PCR using Promega Wizard PCR preps DNA purification kit (Promega Corporation, Madison, WI, USA). The mixture for quantitation consisted of DNA quantitation buffer, sodium pyrophosphate, NDPK enzyme solution, T4 DNA polymerase and DNA. All these contents were incubated at 37°C for 10 min. Then, 100 μL of Enliten L/L reagent was added. Immediately, the reaction was read using a luminometer. The same steps were done on DNAs of known concentrations provided by the kit, and a standard curve was performed by plotting the readings of the luminometer against the concentrations. Then, the readings of the amplified PCR product of PPARß/δ after using the luminometer were read from the standard curve. The results were expressed as (pg/gm tissue) [8].

Statistical analysis

Data were statistically described in terms of range, mean ± standard deviation (± SD), median, frequencies (number of cases) and percentages when appropriate. Comparison of quantitative variables between the study groups was done using the Mann Whitney U test for independent samples. Within group comparison of quantitative variables was done using Wilcoxon signed rank test for paired (matched) samples. For comparing categorical data, Chi square (χ^2) test was performed. Exact test was used instead when the expected frequency was less than 5. Correlation between various variables was done using Spearman rank correlation equation for non normal variables. A probability value *(p value)* less than 0.05 was considered statistically significant. All statistical calculations were done using computer programs Microsoft Excel 2003 (Microsoft Corporation, NY, USA) and SPSS (Statistical Package for the Social Science; SPSS Inc., Chicago, IL, USA) version 15 for Microsoft Windows.

Results

All patients (n=24) completed the full duration of our study (ten weeks). At baseline evaluation, there was no statistical significant difference (p value>0.05) between the two groups regarding either demographic data or clinical parameters *(table 1)*.

In group A (MTX group), the mean PASI was 12.72±7.37 showing no statistically significant difference with group B (PUVA group) where it was10.11±4.78 (p value 0.603). Ten weeks after therapy, both groups showed a significant decrease in PASI score (p value 0.002), with no significant difference between the two groups (4.9±2.58 and 5.77±4.13 in groups A and B respectively) (p value 0.795). However, the mean percentage of reduction in PASI score for group A (59.3±8.26%) was significantly higher than that for group B (42.9 ±19.61%) (p value 0.021).

The levels of PPAR β/δ mRNA were significantly elevated in all patients before treatment (2,773-5,625 μg/gm with a mean of 4,347.71±877.948 μg/gm) compared to those of the controls (314-874 μg/gm with a mean of 66.33±131.651 μg/gm) (p value <0.001). Ten weeks after treatment, PPAR β/δ mRNA significantly decreased in all patients (2,110-4,754 μg/gm with a mean of 3,176.17±831.569) (p value <0.001), however, post treatment levels were still significantly elevated in comparison with those of controls (p<0.001) *(table 2)*.

The level of PPAR β/δ in group A (3,054-5,255 μg/gm with a mean of 4545.00±759.122) was significantly reduced after treatment (2,155-4138 μg/gm with a mean of 3288.33±805.651) (p value 0.002) *(table 3*; *figures 2, 3)*.

In group B, the level of PPAR β/δ (2,773-5,626 μg/gm with a mean of 4150.42±974.918) was also significantly reduced after treatment (2,110-4,754 μg/gm with a mean of 3,064.00±877.179) (p value 0.002) *(table 3*; *figures 2, 3)*.

The mean drop of PPAR β/δ was significantly greater in group A (1,256.66±861.33) compared to group B (1,086.41±626.61) (p<0.05).

In all patients, the PPAR β/δ mRNA level showed no correlation with the PASI score either before or after treatment (r 0191, p 0.373 and r 0.173, p 0.418 respectively). Also, the decrease in PPAR β/δ mRNA level did not correlate with the reduction in PASI score in either group (r 0.206, p 0.52 and r 0.028, p 0.377 in groups A and B respectively). However, the PPAR β/δ mRNA level before treatment showed a significant positive correlation (r 0.419, p 0.041) with the duration of psoriasis.

Table 1. Summary of baseline data of patients

Patients	Age	Sex	Disease duration	Pre treatment PASI Score	Pre treatment PPAR β/γ mRNA (μg/gm)
Group A (methotrexate)	18-60 (41.58± 14.935)	M: 10 (83.3%) F: 2 (16.7%)	2-20 (6.92± 7.113)	3.1-23.8 (12.717± 7.3669)	3,054-5,255 (4,545.00± 759.122)
Group B (PUVA)	15-56 (13.67± 12.22)	M: 8 (66.7%) F: 4 (33.3%)	1-12 (6.50± 3.802)	3.6-18.4 (10.133± 4.7721)	2,773-5,626 (4,150.42± 974.918)
P value	>0.05				

P value <0.05 is significant.

Table 2. PPAR β/γ mRNA levels in all patients before treatment and controls.

	All patients before treatment	All patients after treatment	Controls
PPAR β/γ mRNA (μg/gm)	2,773-5,625 (4,347.71±877.948)[a,c]	2,110-4,754 (3,176.17±831.569)[b,c]	314-874 (66.33±131.651)[a,b]

[a,b] Significant p<0.001 comparing patients before and after treatment respectively to controls. [c] Significant p<0.001 comparing patients before and after treatment.

Table 3. PASI scores and PPAR β/γ mRNA levels in patients 12 weeks after therapy.

Group	PASI Score before therapy	PASI Score after therapy	PPAR β/γ before therapy (μg/gm)	PPAR β/γ after therapy (μg/gm)
Group A (methotrexate)	3.1-23.8 (12.717± 7.3669)	2-9.5 (4.892±2.5787)	3,054-5,255 (4,545.00± 759.122)	2,155-4,138 (3,288.33±805.651)
P value	0.002		0.002	
Group B (PUVA)	3.6-18.4 (10.133± 4.7721)	2.1-17.1 (5.767±4.1307)	2,773-5,626 (4,150.42± 974.918)	2,110-4,754 (3,064.00±877.179)
P value	0.002		0.002	

P value <0.05 is significant.

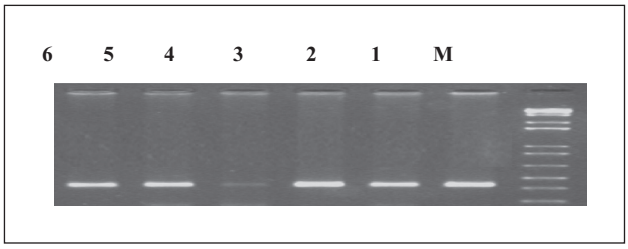

Figure 2. Agarose gel electrophoresis 2% stained with ethidium bromide showing gene expression of PPAR β/δ at 287 bp. Lanes 1, 3, 5: cases of psoriasis before treatment with methotrexate.
Lanes 2, 4, 6: same cases after treatment.
M: molecular DNA marker.

Discussion

The possible role of PPAR β/δ in psoriasis was supported in our study by the detection of significantly elevated levels of PPAR β/δ mRNA in psoriatic patients compared to the levels measured in the controls (p value<0.001) and its significant reduction after treatment with MTX or PUVA. In agreement with our findings, PPAR β/δ expression was reported to be dramatically increased in the hyperproliferative lesional skin of psoriatic patients [8-10]. It has also been reported that PPAR β/δ gene expression is upregulated in mouse skin in response to inflammatory cytokines [11], suggesting that the increased expression in psoriatic lesions is most probably due to pro-inflammatory signals. In addition, numerous lipid molecules, such as lipooxygenase products, which are potent activators of PPARs in human keratinocytes, accumulate in psoriatic lesions [9], which could be a reason behind their elevated levels in psoriasis.
The consequences of PPAR β/δ activation in this pathological situation are unclear [11]. It has been recently proven that activation of PPAR β/δ in the epidermis is in itself sufficient to trigger inflammatory changes, immune activation and signaling, as well as gene dysregulation characteristic of psoriasis [4]. One mechanism by which PPAR β/δ may play a role in the pathogenesis of psoriasis was suggested by Yacoub *et al.* [6] who documented that PPAR β/δ enhances the proliferation of T cells and blocks their apoptosis, leading to the persistence of activated T cells in psoriatic lesions, the hallmark of psoriasis. Moreover, PPAR β/δ has a questionable role in inducing angiogenesis as Nijsten *et al.* [12] showed that microvessel density was significantly higher in actinic keratosis and squamous cell carcinoma expressing PPAR β/δ. The fact that psoriasis has recently been recognized as an angiogenesis-related disease [13] raises another possible attributing role of PPAR β/δ in the pathogenesis of psoriasis.

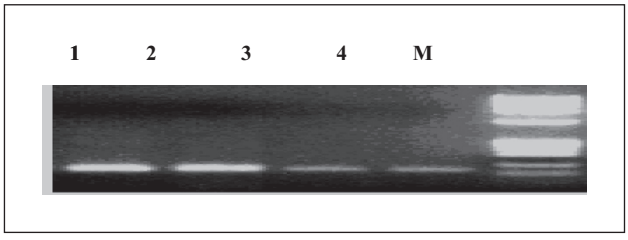

Figure 3. Agarose gel electrophoresis 2% stained with ethidium bromide showing gene expression of PPAR β/δ at 287 bp.
Lanes 1-2: cases of psoriasis before treatment with PUVA.
Lanes 4 6: same cases after treatment.
M: molecular DNA marker.

In the present study, both MTX and PUVA groups showed a significant reduction in PPAR β/δ levels ten weeks after treatment, but failed after this period to normalize their levels, which remained significantly higher than those of the controls. The reduction was significantly greater in the MTX group in comparison to the PUVA group. Also, the reduction of the PASI score was found to be significantly higher in the MTX group, documenting the superiority of MTX in the treatment psoriasis, in agreement with other studies [14], and we further document it having a more pronounced effect on the reduction of PPAR β/δ in psoriasis.
It is still debatable whether the clinical improvement achieved by these classical therapeutic modalities was partially due to their direct effect on the alteration of the PPAR β/δ levels, or if the reduction of the PPAR β/γ levels came as a sequel of the improvement of the psoriasis, by other mechanisms of action. This debate is evident for several reasons.
First: Both modalities exert their effect mostly on lymphocytes, and though different mechanisms might operate for psoriasis, T lymphocytes play a central role in the events leading to development of psoriatic lesions [15]. It was suggested before by Jeffes *et al.,* [16] that proliferating lymphoid cells are more likely than epithelial cells to be a cellular target of MTX in psoriasis [16]. This has been further documented by Rentenaar *et al.* [17] who demonstrated that a decrease in circulating skin homing T cells is responsible for part of the therapeutic effect of MTX in severe psoriasis. Similarly, it is well known that PUVA's mechanism of action in psoriasis stems from its toxic effects on activated lymphocytes [7]. Thereby, it could be speculated that both MTX and PUVA either counteract the pro-survival influence of PPAR β/δ on T lymphocytes, leading to the reduction of T lymphocytes and thereby an improvement of the psoriatic lesions, or directly inhibit the T lymphocytes by other mechanisms, leading to a reduction in PPAR β/δ levels and improvement of psoriasis.
Second: Both MTX and PUVA exert anti-inflammatory effects. It has been proved that MTX reduces the activation state of antigen-stimulated cells, alters the expression of adhesion molecules [18] and reduces tumor necrosis factor-α with its consequent inflammatory reactions [19]. Also, PUVA has been shown to reduce lymphocytes, macrophages, dendritic cells and epidermal Langerhans cells in psoriatic lesions, as well as effectively diminish the expression of CD86 and inhibit this step of inflammation [15]. Accordingly, both MTX and PUVA would presumably lead to downregulation of the pro-inflammatory signals which upregulate the PPAR β/δ expression [11]. In this case, favoring the possibility that the reduction of PPAR β/δ came as a sequel of the improvement of psoriasis.
Third: Both MTX and PUVA exert antiangiogenic effects. Several studies have reported that MTX has an antiangiogenic activity [20]. Similarly, photochemotherapy has been shown to inhibit angiogenesis and induce apoptosis

of endothelial cells [21]. This antiangiogenic effect of MTX and PUVA may be partially through antagonizing the proangiogenic role of PPAR β/δ, eventually leading to the improvement of psoriasis.

In the current study, PPAR β/δ mRNA, despite markedly decreasing after ten weeks of therapy, remained significantly elevated in comparison with controls ($p<0.001$). This could be explained by the fact that the patients did not achieve a complete cure, with the average PASI score being 2-9.5 and 2.1-17.1 in groups A and B respectively after therapy. Therefore an extension of the treatment period until complete recovery is achieved is recommended for proper evaluation of the effect of MTX and PUVA on PPAR β/δ mRNA levels.

The fact that the PPAR β/δ mRNA levels in our study had a positive correlation with the disease duration ($r = 0.173$, $p = 0.419$), points to a possibly more important role of PPAR β/δ in the maintenance of the disease rather than its initiation. On the other hand, the absence of a correlation between the PPAR β/δ levels and the PASI scores suggests that PPAR β/δ levels do not reflect disease severity. Also, the absence of a correlation between the reduction of PPAR β/δ levels and the reduction of PASI scores in both groups may be attributed to the fact that reduction of PPAR β/δ levels is not the only mechanism by which MTX and PUVA exert their therapeutic effects in psoriasis.

In conclusion, our prospective comparative controlled study conducted on 24 psoriatic patients documents the possibly important role of PPAR β/δ in the pathogenesis of psoriasis and raises its possible role in the maintenance of long standing lesions, not however, reflecting the severity of the condition. We further showed that the standard therapeutic modalities, methotrexate and PUVA, constitute highly effective therapeutic modalities for psoriasis which might exert their anti-psoriatic activities partially by altering the expression of PPAR β/δ, either directly or indirectly, through inhibition of T lymphocytes and proinflammatory cytokines. ■

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