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Non-aromatic naphthalane preparation; preliminary clinical study in the treatment of psoriasis vulgaris

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Abstract

This study aimed to prove the similarity of the composition of non-aromatic Croatian naphthalane (NAN) with brown naphthalane (BN), which is used in the treatment of psoriasis vulgaris. The comparison of the compositions was performed by obtaining GC fingerprints, which were supported by GC-MS data. In spite of remarkable differences in general profiles of the GC chromatograms, lower and medium molecular weight components of NAN were found to be qualitatively the same as the saturated constituents of BN. Quantitatively, lower molecular weight components as well as all *n*-alkanes were comparatively lower in NAN. NAN, additionally, contained higher molecular weight components, among which there were saturated oligocyclic hydrocarbons (up to pentakishomohopanes), described as responsible for the curing effect of naphthalane. The composition characteristics of NAN including its non-aromatic character made it suitable for a clinical study. In the treatment, the efficacy was determined by means of comparison of Psoriasis Area Severity Indices, PASI, at the beginning and at the end of the therapy. Adult volunteer-patients, nine males and six females, applied NAN over the whole body, except the scalp, at the room temperature for 20 min and this was followed by the selective UVB radiation. After the 3-week therapy, all essential clinical manifestations as erythema, desquamation and infiltration were significantly reduced in 14 patients; in nine cases the improvement was 50–93%, while the state of five patients improved between 25 and 50%. In one case, there was no obvious change. No exacerbation occurred during the therapy period. No adverse effect on hematological or biochemical parameters was noticed.

Keywords: Croatian naphthalane petroleum; Naphthalane healing preparations; Psoriasis Area Severity Index, PASI; Petroleum preparations; Psoriasis vulgaris; Skin diseases; Skin treatment

1. Introduction

Naphthalane preparations are applied in a wide range of medical indications [1]. Encouraging re-

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sults were reported in the diminishing of skin lesions by application of brown naphthalane to patients suffering from psoriasis vulgaris [2].

Psoriasis vulgaris [3,4] is a recurrent chronic disease, affecting 1–3% of the human population. At dermatological clinics, psoriatic patients make up 6–8% of those attending. The etiology of the disease has not been completely elucidated yet. Two patterns: early-onset and late-onset types have been differentiated according: to their genetic characteristics, to the patient's age and to the course of the clinical disease. The basic pathogenesis mechanisms of psoriasis may be summarized as epidermal hyperregeneration and disturbance in keratinization followed by infiltration of inflammatory components into the skin, causing self-perpetuating lesions resulting from: erythema, desquamation and infiltration.

Since psoriasis vulgaris is a chronically recidivous disease, lasting for the rest of the patient's life, therapeutic means should be chosen carefully, in order to minimize the accumulation of negative side-effects throughout long-term therapy. For that reason, the chemistry of naphthalane preparations requires further research as does the procedures used for the delivery of the treatment in order to minimize adverse effects.

Regarding the chemical composition of naphthalane preparations, the following should be taken into consideration:

- the method of preparation should conserve the structures of the constituents so that they remain identical with those occurring in the curable petroleum used as a raw material [5];
- the preparation should be rich in saturated oligocyclic hydrocarbons (especially in steranes [6]) because of their bioactivity [7]; and
- since among aromatic compounds there are carcinogens, aromatics should be avoided as constituents, and preparation should obey the contemporary pharmacopoeias [8].

A previously studied non-aromatic naphthalane preparation (P3 in [5]) was found to satisfy the above criteria. In the current paper, its efficacy in the treatment of psoriasis vulgaris was studied. To the authors' knowledge, such a non-aromatic naphthalane preparation has not been available before and this was its first clinical study.

Prior to its acceptance for a preliminary clinical study, the non-aromatic naphthalane (NAN) preparation had to be proved to be of similar or improved chemical composition in comparison with brown naphthalane (BN). BN has been in daily use (in the Naftalan–special hospital for rehabilitation, Ivanić-Grad, Croatia; the Hospital) in application (to more than 9700 patients) for several indications, including psoriasis vulgaris [2]. For comparison reason, the chemical composition of NAN was studied in details (remarkably more than published previously [5]) and it was carefully compared to BN composition.

In the Hospital, the BN therapy involves immersing the patient in a tub at 34–37 °C. Apart from the head, the whole body is submerged in BN for 12–14 min. After bathing in BN, patients are exposed to selective UVB radiation followed by a mild shower and application of a neutral ointment.

2. Chemical investigation

2.1. Experimental

The NAN sample was available in the quantity of 30 l. Its characteristics have been described elsewhere [5]. BN was declared to have a density (ρ) at 15 °C (ASTM D 1298): $\rho = (0.9173 \pm 0.0002)$ g/cm^3; with a refractive index ($n^{20}{}_D$) (ASTM D 1218): $n^{20}{}_D = 1.5085 \pm 0.0002$; and with an elemental composition: $C_{\%mass} = (87.9 \pm 0.3)$ and $H_{\%mass} = (12.1 \pm 0.1)$. It has a brown colour and a smell like gasoline.

NAN and BN were compared by means of gas chromatography (GC) under the following conditions: instrument, Pye Unicam 304; column fused-silica, DB-1 (30 m l × 0.25 mm i.d. × 0.25 μm film thickness), injection, split mode; carrier gas, hydrogen; detection, flame ionization; temperature program, start 100 °C, temperature increase 5 °C/min, final 310 °C for 30 min. Under the same conditions, GC chromatogram of a reference mixture of *n*-alkanes was analysed in order to determine the top distillation temperature of NAN.

For reasons of comparison, aromatics were re-

moved from BN, by adsorption on a silica-gel column (480 mm l × 5 mm ϕ) followed by elution of non-aromatic compounds with *n*-pentane until the aromatics started eluting, monitored by an UV lamp.

Mass spectra were obtained using a gas chromatograph–mass spectrometer (GC–MS) under the following conditions: GC (Varian 3700); column, fused-silica, DB-1, 60 m l × 0.25 mm i.d. × 0.25 μm film thickness; temperature program, start 50 °C, temperature increase 6 °C/min up to 280 °C; carrier gas, helium; GC–MS coupling, direct inlet; MS (Varian MAT 112S), scanning rate, 1 s per decade; interscan time, 0.2 s; ionization, electron impact, electron energy, 70 eV; emission current, 0.7 mA; resolution, 1:600; *m/z* range, 50–500; ion source temperature, 260 °C; ion source pressure, 10^{-5} Pa.

2.2. Results and discussion of chemical investigation

The GC chromatogram of NAN revealed an unresolved complex mixture containing an extremely high number of GC peaks, many of them highly overlapping (Fig. 1(a)). The complex composition including a number of homologues and isomers, as well as numerous unknowns, made it impossible to compare the composition of the NAN and the BN sample on the basis of identification the each single component. Thus, as the comparison base, GC fingerprints supported with GC–MS data were used.

The GC chromatograms of NAN and BN (Fig. 1(b)) differed widely. While NAN contained large amounts of higher molecular weight constituents, BN was relatively rich in lower molecular weight

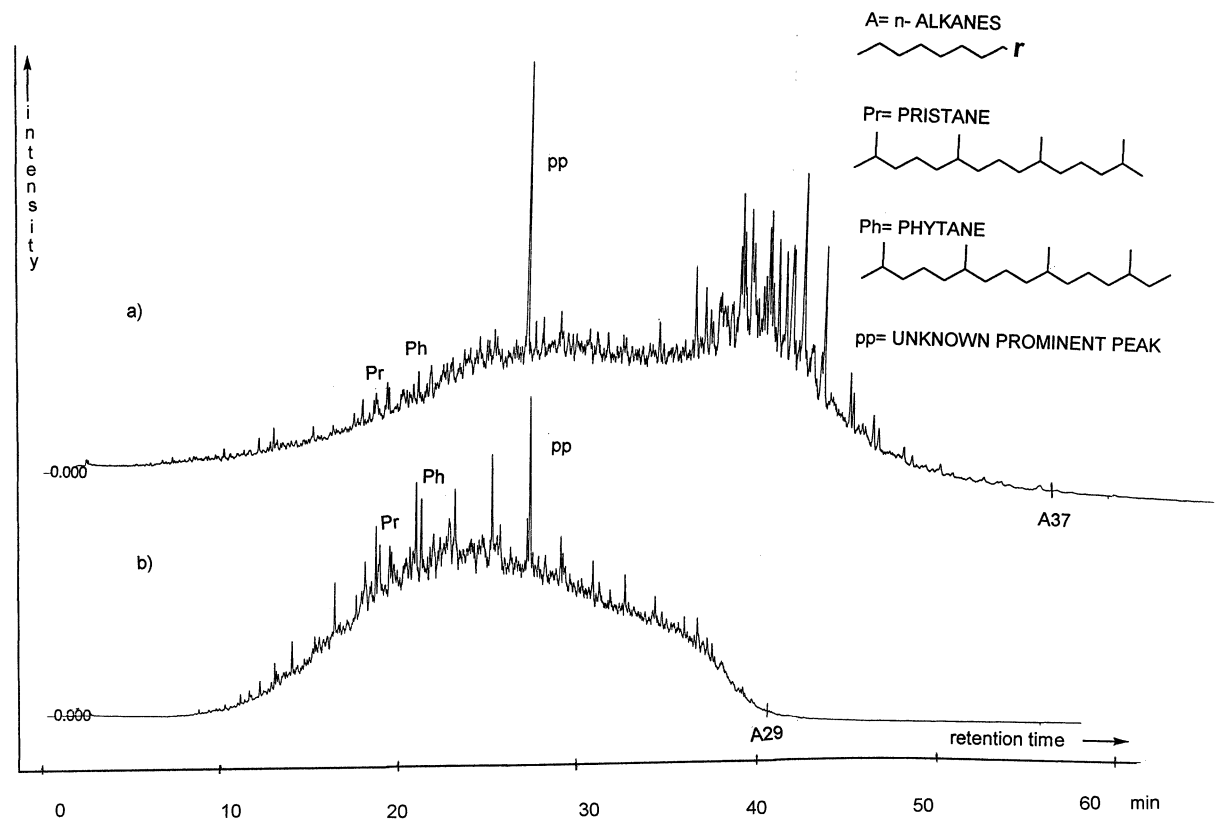

Fig. 1. GC chromatograms of NAN (a) and of the whole BN sample (b). A number beside a letter relates to the C-atom number in the molecule. A37 and A29 mark the place where *n*-$C_{37}H_{76}$ (T_{bp} = 503 °C) and *n*-$C_{29}H_{60}$ (T_{bp} = 440 °C) elute under the same GC conditions.

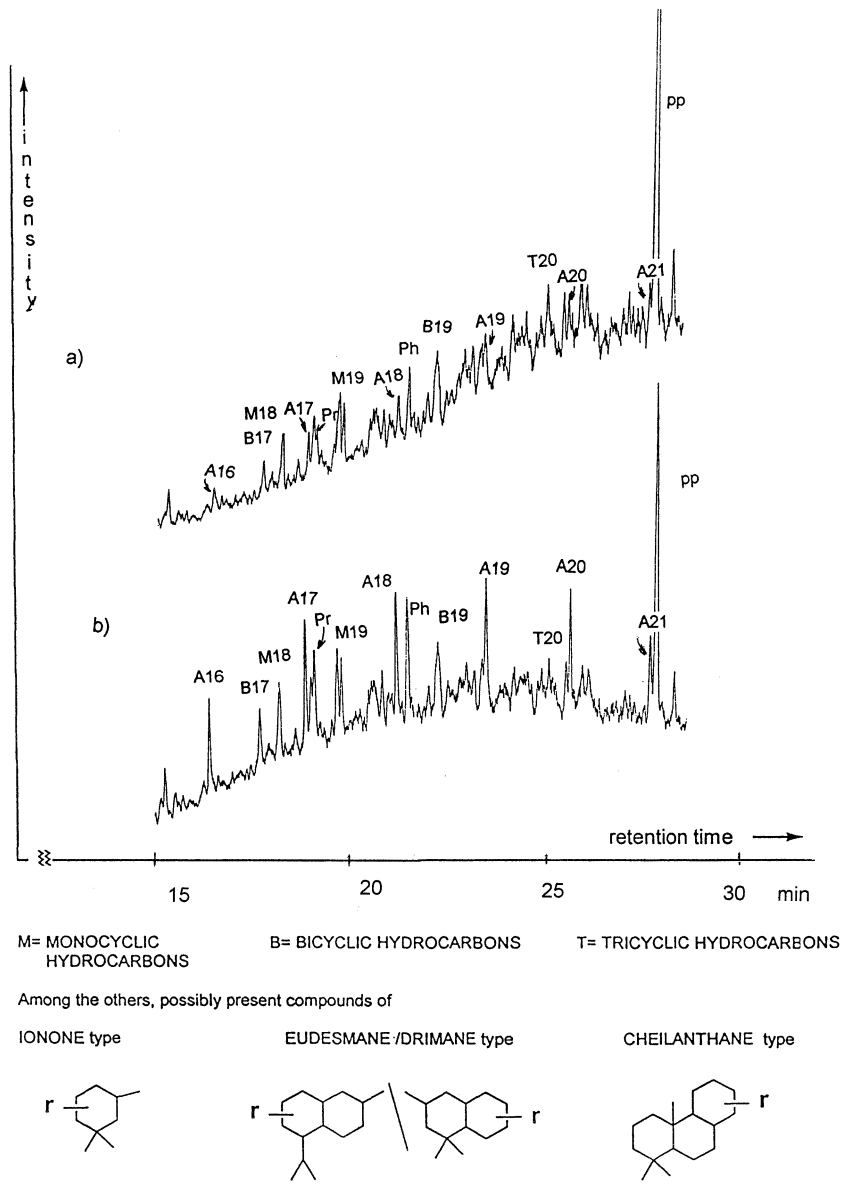

Fig. 2. Comparison between the lower molecular weight parts of the GC fingerprints of NAN (a) and BN′ (b). Letters are as in Fig. 1 and as explained in the legend beneath.

part. The homologous series of *n*-alkanes (in Fig. 2 labelled by A) was easy to observe in BN while in NAN it was rather weak. Also, the geogenic isoprenoids pristane (Pr) and phytane (Ph) were found to be comparatively low in NAN.

In spite of differences in general profiles of the GC chromatograms, high degree of similarity in qualitative composition was found, when comparing NAN and the dearomatized BN fraction, BN′. MS spectra of the peaks in the chromatograms, confirmed the presence of the same (presumably all) components in NAN as constituted BN′. There was a variety of cyclic and non-cyclic saturated hydrocarbons. As an illustration, in Fig. 2

lower molecular weight parts of the NAN and BN′ fingerprints are shown. Labelled peaks relate to some acyclic (A, Pr and Ph) and cyclic hydrocarbons. Among the latter ones, there were found to be monocyclics (M), bicyclics (B) and tricyclics (T). According to petroleum geochemistry [10], among the other cyclic moieties, the ionone type for M, the drimane/eudesmane type for B and the cheilanthane moiety for T, might be expected (possibly all with the attached alkyl chain/s).

Both chromatograms possessed the same peak marked as pp. The medium molecular weight part of NAN chromatogram corresponded to the final part of BN′ chromatogram. In these sequences, the constituents of NAN and BN′ also corresponded. Regarding the chemical composition, both for NAN and BN′ they belonged to the similar classes of compounds (with increasing molecular masses) as appeared in the beginning parts of chromatograms and were, additionally, accompanied with tetracyclics (of sterane and non-sterane type). If the relative heights of A, Pr and Ph are neglected, their GC peaks are found to be arranged in a similar pattern.

According to the GC retention data (regarding the homologous series of reference *n*-alkanes), NAN was found to distil in a range almost reaching 500 °C while BN distilled in a range roughly up to 440 °C. A relatively high top boiling point temperature of NAN was confirmed by the presence of hopanes, which are also known as genuine petroleum oligocyclics [9]. They were identified by extracted ion chromatograms (mass fragmentograms) (Fig. 3) $m/z = 191$ for the hopanes principal fragment ion, and $m/z = 412 + n \times 14$ for the hopanes parent ions. Their presence (in the homologous range up to pentakishomohopanes S and R) should be recognized as an advantage of NAN. Besides contributing to the common amount of the saturated oligocyclic hydrocarbons, the hopanes indicated the top distillation temperature of NAN to be high enough to provide the complete presence of geogenic steranes. The majority of petroleum steranes (nor-cholestanes, cholestanes, ergostanes, stigmastanes and n-propyl-cholestanes) elute from the GC column preceding hopane $C_{30}H_{52}$ [9] (and distil in a comparatively lower temperature range). Natural geogenic variety of steranes of genuine petroleum origin in NAN, have been discussed earlier [5].

3. Preliminary clinical investigation

3.1. Methodology

The preliminary clinical investigation was comprised of 15 volunteers; nine males and six females, in the age range from 20 to 61 (Table 1), all with the confirmed diagnosis of psoriasis vulgaris. (Patients suffering from diabetes, hepatic, urologic or cardiovascular diseases were not included. There were no patients with neurological disorders or with a tendency to neuralgic excitation. No pregnant or nursing women were included.) Within this group of patients, the disease duration ranged from 3 to 34 years. All of them had psoriatic skin changes including erythema, infiltration and desquamation. Except for two patients, they had been previously treated with BN, 1–9 times. (Before starting the therapy with NAN, the patients had taken no topical steroids for at least two weeks, or systemic therapy, which could include corticosteroids, retinoids or cyclosporin, for at least 3 months.) The treatment was performed in an outpatient clinic mode.

The limited quantity of the NAN preparation available (30 l) demanded modification of the therapy compared to the one practised daily with BN. Instead of bathing the patients in warm tubs, 100 ml of NAN were applied onto the entire skin, except for the scalp, at room temperature for 20 min. The rest of the treatment was analogous to the therapy with the BN preparation Section 1. Patients were exposed to the selective UVB radiation (total average per a patient 7.74 J/cm^2) by Psorilux 5050 (Heraeus, Hanau, Germany). A mild shower and application of neutral Belobaza® ointment followed. The procedure was taken once a day, six times per week, for 3 weeks.

In order to objectify the evaluation of the therapic efficacy, the psoriasis area severity index (PASI) [10] was used. PASI is based on observation of the erythema, infiltration and desquamation at the patient's head, trunk, and upper and

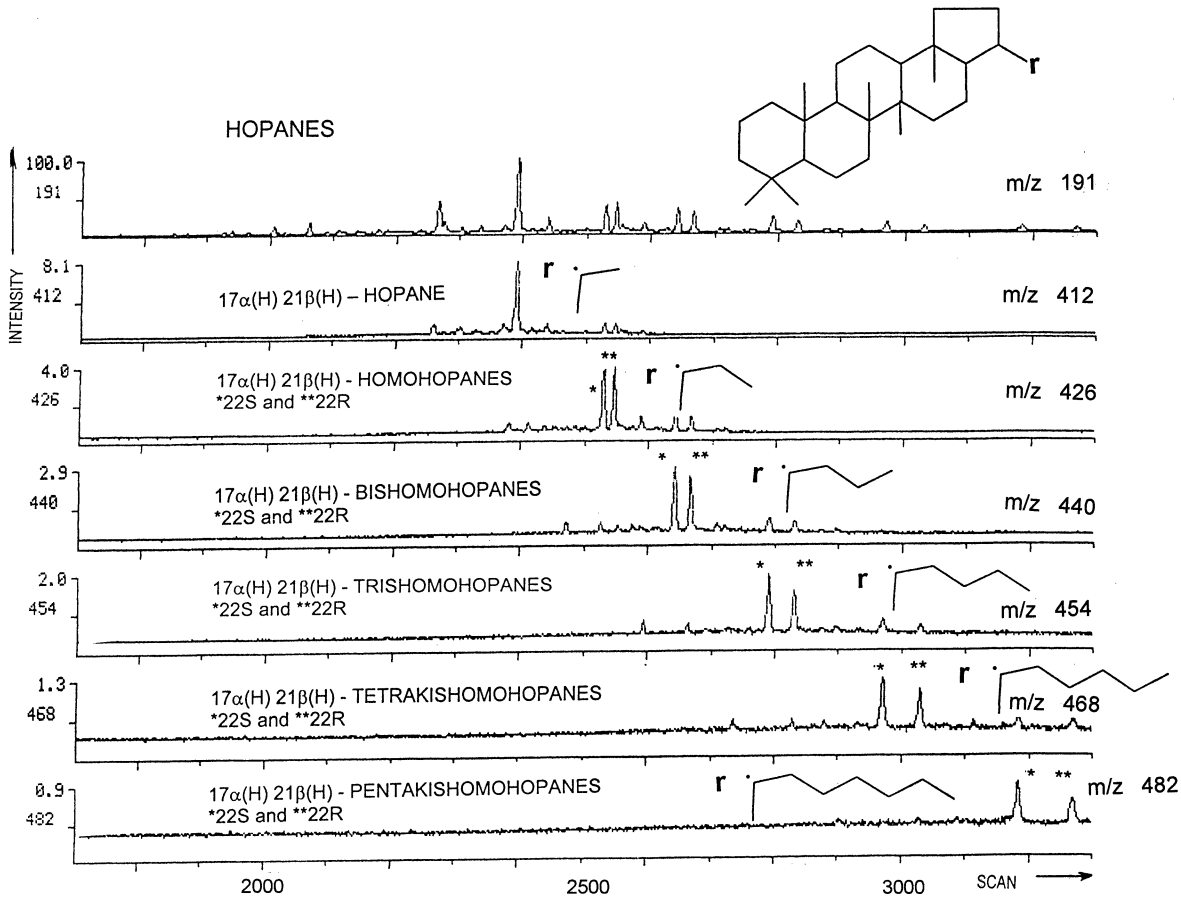

Fig. 3. Extracted ion chromatograms of hopanes $m/z = 191$ for hopanes, generally; and for the members of homologous series: $m/z = 412$ for 17α (H) 21β(H) hopane with $r = {}^{\bullet}C_3H_7$; $m/z = 426$ for 17α (H) 21β(H) homohopanes 22S and 22R with $r = {}^{\bullet}C_4H_9$; $m/z = 440$ for 17α (H) 21β(H) bishomohopanes 22S and 22R with $r = {}^{\bullet}C_5H_{11}$; m/z 454 for 17α (H) 21β(H) trishomohopanes 22S and 22R with $r = {}^{\bullet}C_6H_{13}$; $m/z = 468$ for 17α (H) 21β(H) tetrakishomohopanes 22S and 22R with $r = {}^{\bullet}C_7H_{15}$, and $m/z = 482$ for 17α (H) 21β(H) pentakishomohopanes 22S and 22R with $r = {}^{\bullet}C_8H_{17}$.

lower extremities. The severity of the attack and the area of the affected skin are estimated pseudo-quantitatively. Each body part is taken into account in proportion, approximately, as participating in the total skin area. PASI ranges between 0 (no obvious skin changes) and 100 (entire skin severely attacked by psoriatic changes). Statistical analysis of PASI scores was performed before and after the therapy using the Student's t-test for dependent samples.

For the ethical reasons, there was no control group receiving only UVB radiation since no significant improvement would be expected.

3.2. Results and discussion of the preliminary clinical investigation

During the 3-week therapy with NAN, no exacerbation occurred. The therapy resulted in reduction of all clinical psoriatic skin changes in 14 patients; total regression of desquamation occurred, while erythema and infiltration reduced significantly. In one case, no improvement was observed.

In nine cases PASI dropped by 50–93% and in five patients it decreased for 25–50% (Table 1, Fig. 4). In one case, PASI remained the same. The

Table 1
Patients list and PASI scores before and after the NAN therapy

No.	Patients	ID	Sex	Age years	Suffering for years	No. Th[a]	PASI[b]	$PASI_{rest}$ (%)	$PASI_{score}$ (%)
1	M.K.	K1051	M	31	3	First	2.6/14.1	18.4	81.6
2	M.P.	P1292	M	20	15	First	7.2/21.6	33.3	66.7
3	B.M.	M110	M	46	20	Seventh	9.4/28.4	33.1	66.9
4	I.P.	P7	M	61	34	Eighth	6.0/19.2	31.3	68.7
5	A.B.	B608	M	53	10	Fourth	8.8/18.2	48.4	51.6
6	M.J.	J49	F	60	10	Sixth	3.0/6.0	50.0	50.0
7	Z.Z.	Z16	M	38	21	Seventh	18.2/26.5	68.7	31.3
8	G.C.	C129	M	30	7	Third	11.6/15.6	74.4	25.6
9	J.C.	C59	M	42	10	Third	13.3/19.2	69.3	30.7
10	M.M.	M941	M	36	2	Eighth	15.4/23.7	65.0	35.0
11	M.F.	F282	F	43	20	Tenth	21.1/44.5	47.4	52.6
12	D.K.	K229	F	44	7	Fourth	2.4/11.0	21.8	78.2
13	M.D.	D1	F	42	20	Fourth	11.1/17.1	64.9	35.1
14	A.S.	S637	F	56	20	Second	1.4/20.1	7.0	93.0
15	A.M.	M29	F	42	7	Second	43.1/43.1	100.0	0.0

[a] No. Th = number of the BN therapy earlier applied plus this therapy with NAN.
[b] Values of the PASI at the end/the PASI at the beginning of the NAN therapy.

average improvement was found to be 51.1%, when calculated for all patients and 54.8%, when the patient with no improvement (no. 15) was excluded from the calculation. The Student's *t*-test for dependent samples revealed that decrease of PASI scores was statistically significant ($P = 0.000028$) and that mean PASI score, which was 21.89 ($\pm$ 10.53) prior to the treatment, decreased to 11.64 ($\pm$ 10.52) after 3 weeks of therapy.

According to the Hospital's statistics [3] with the BN therapy in warm tubs, PASI was, on average, decreased by cca. 70% (when BN was applied to 30 hospitalised patients, PASI decreased by 67%; two exacerbated patients were excluded during the therapy and from statistical calculation).

A comparatively higher PASI efficacy for BN should be explained by therapy conditions offering more intense contact patient-to-preparation in tubs at the body temperature, as well as the patients' hospitalization.

Biochemical (fasting blood sugar, uric acid, creatinine, cholesterol, triglycerides, AST, ALT, γGT and protein in urine) and hematological (red blood cells count, hemoglobin, white blood cells count, lymphocytes and erythrocyte sedimentation rate) laboratory parameters in four randomly chosen patients indicated no adverse effect resulting from the applied NAN therapy. Thus, it may be considered that the topical NAN application provoked neither biochemical nor hematological negative changes. The results of the measurements of biochemical and hematological parameters were taken as an indication of no adverse effect of NAN therapy on bone marrow, liver and kidney.

4. Conclusion

According to the chemical investigation, the non-aromatic naphthalane preparation NAN was found to be comparable in its low and medium molecular weight parts to the saturated components of brown naphthalane BN (NAN comprised presumably all the saturated BN constituents). In addition to the absence of aromatics, as a marked composition difference, the presence of hopanes and an abundant amount of steranes were found in NAN. The differences were thought to be in favour of NAN, thus it was found to deserve a preliminary clinical study of its efficacy in the treatment of psoriasis vulgaris.

The limited quantity of the NAN sample demanded some therapic modifications compared to the BN therapy, which narrowed the scope for

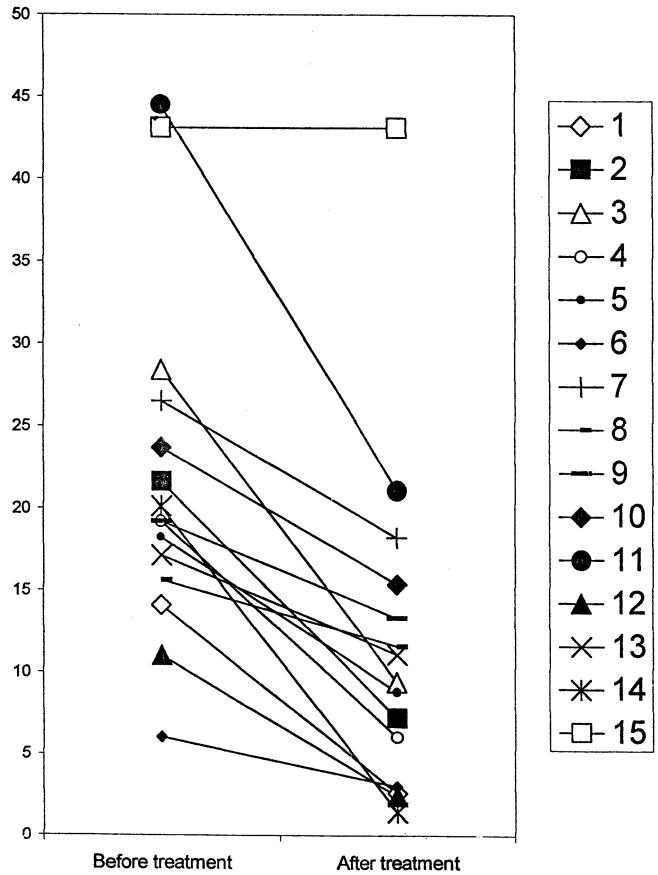

Fig. 4. PASI before and after 3-week therapy with NAN. The numbers in the legend on the right side are related to the ordinal numbers in Table 1.

exact comparison of the results. Nevertheless, the preliminary study of topical application of NAN to psoriasis vulgaris afforded good results. All the essential psoriatic clinical manifestations such as erythema, desquamation and infiltration were significantly reduced in 14 of 15 treated patients. For them, PASI decreased in the range 25.6 up to 93.0%, on average for 54.8%. In one case there was no change (average for all patients 51.1%). The Student's t-test found the PASI score decrease to be statistically significant. During the therapy, in no case exacerbation occurred. No side effects were observed.

According to the laboratory data, NAN caused no adverse effect on hematological and biochemical parameters. Thus, it might be considered not to be hematotoxic, nephrotoxic or hepatotoxic.

The preliminary results presented in this paper indicate that NAN seems to be a promising potential natural medium for psoriasis vulgaris treatment. It deserves further investigation.

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