Journal of Pre-Clinical and Clinical Research, 2015, Vol 9, No 2, 120-123
www.jpccr.eu

ORIGINAL ARTICLE

Relations between excretion of indole melanogen (TPM) and time of exposure to solar radiation

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Kubiński Z, Kwapuliński J, Brewczyński P, Roczniak W, Babuśka-Roczniak M. Relations between excretion of indole melanogen (TPM) and time of exposure to solar radiation. J Pre-Clin Clin Res. 2015; 9(2): 120–123. doi: 10.5604/18982395.1186491

Abstract

Introduction. In the human skin exposed to ultraviolet (UV) radiation, melanogenesis occurs in two stages, accompanied by urinary excretion of Thormalen-positive melanogen (TPM). In Poland, no data are available on the course and intensity of melanogonesis in relation to UV exposure in an industrial region.
Material and methods. The Thormalen test was used for the collected samples (N=136) as modified by Matous and Suchoń.
Results. Maximum environmental TPM content (0.67 μg/dm^3) was observed in August.
Conclusion. The time of UV exposure and local type of solar radiation promote melanogenesis.

Key words

melanogenesis, Thormalen test, solar radiation, skin

INTRODUCTION

The process of stimulation of melanin skin pigmentation entails two processes:

1) immediate pigment darkening (IPD) and 2) delayed pigment darkening, also referred to as melanogenesis (Fig. l). The IPD reaction is specific to each individual. The minimum dose of solar radiation which evokes this reaction ranges from 1–2.5 J per square centimeter of skin. IPD is a fast and low-threshold reaction that can be induced within a few minutes [1, 2, 3]. The wavelengths below 320 nm that cause sunburn (erythematous effect) are considered to stimulate melanogenesis. However, UV radiation ranging between 550–450 nm has the greatest effect on the process of melanogenesis in white people, the threshold dose at 560 nm being approximately 1J x cm^2. In Europe, visible radiation can also stimulate melanogenesis [1]. However, the threshold dose and its scope have not been defined, as the values vary considerably in the population. For monochromatic light of 300 nm wavelength, the threshold dose is 100 mJ × cm^2. Two different photobiological phenomena take place in the human skin after UV exposure:

– instantaneous tan in the form of chromatosis beginning just after or even during solar exposure, to disappear after 1 min – l h;
– delayed tan, which can be caused by exposure to both long-wave UV and visible radiation.

In Figure 1, the two processes in melanin pigmentation stimulated by sunlight are illustrated by the dotted line:

a) immediate pigment darkening (IPD) or immediate tanning response;
b) melanogenesis or delayed pigment darkening.

The solid line curve represents the course of erythema response 1.

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Received: 08 February 2015; accepted: 09 September 2015

The intensity of skin erythema due to solar radiation depends on the content of melanin – the skin pigment, the skin thickness and some environmental factors, including season of the year, time of day, latitude and altitude, which condition UVB radiation intensity in the solar spectrum.

In most cases, chromatoses occur after 2–3 days and may linger up to several months [4]. Skin hyperpigmentation is mainly UVB- and less UVA-dependent, being a defence reaction to the harmful effects of sunrays. Ultraviolet radiation stimulates melanogenesis, the process in which melanin, the major skin pigment, is produced. Its level depends largely on the skin phototype.

According to other data, a single dose of visible radiation of 400–700 nm wavelength and infrared radiation (above 760 nm) induces weak melanogenesis. Repeated exposure to visible or infrared radiation (50–100 J per square centimeter of skin) may stimulate slight melanogenesis [1].

The above data are only of regional significance, due to the varying degree of absorption of the visible part of solar radiation spectrum. Thus, the skin of light blondes reflects 45% of solar radiation energy that reaches the body surface: for the skin of a black-haired people or black persons these values are respectively 35% and 16% [5].

According to Czech scientists, the physiological process of melanogenesis in the human body is as follows [6, 7]:

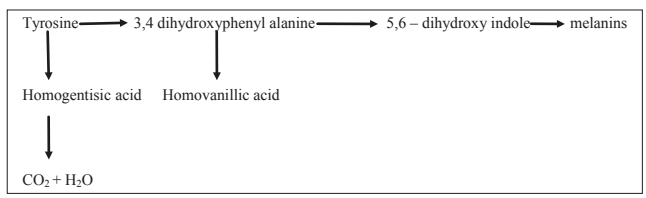

Figure 1. Melanogenesis phenomenon

A survey of literature sources shows the fragmentary nature of the research findings obtained so far. Surprisingly, data are missing on the changes in the level of excreted TPM depending on the time of solar radiation effect on

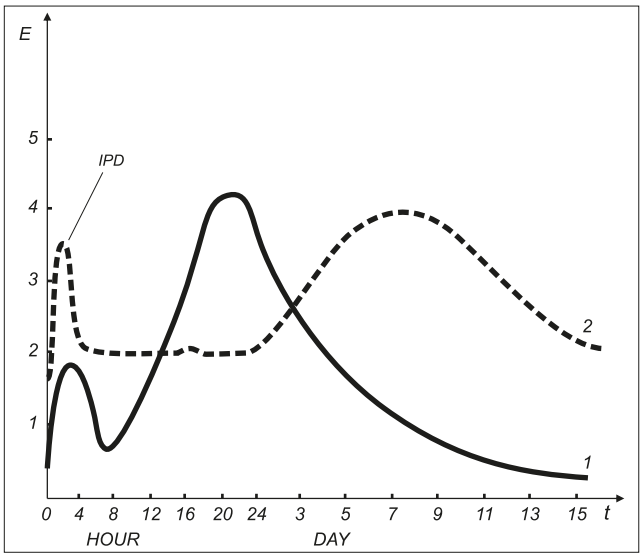

Figure 1. Course of melanogenesis in time.
E – Intensity of response (arbitrary units)
1. Two general processes in melanin pigmentations stimulated by sunlight are illustrated by a dotted line:
   a) immediate pigment darkening (IPD) or immediate tanning response
   b) melanogenesis or delayed tanning reaction
2. The solid line curve represents the course of erythema response 1

the human skin in Poland. Thus, it seemed purposeful to undertake biochemical investigations in order to determine melanogenesis intensity depending on solar radiation characteristics in various seasons of the year (January, April, August, November) in an industrialized region.

MATERIALS AND METHOD

The study used 24-hour urine samples obtained from 134 individuals aged 4–56 years, inhabiting a heavily industrialized region in January, April, August and November in 1990–2010. The study group comprised 17 children aged 4–10 years, 69 adults aged 25–34 years, 41 adults aged 35–45 years, and 7 aged 45–56 years. There were no people between the ages of 10–25. All the study subjects lived in a heavily industrialized region. The study participants aged 25–45 formed the most numerous group (N=100), including 40 who worked outside for at least 6 hours daily, 30 who stayed in the open air for at least 3 hours during the day, and 30 farmers who spent most of the day outside. In the region inhabited by the study subjects, the solar radiation spectrum is determined by fine dust particles from the energy industry, ceramic industry and oil refining. The study participants were on optional diets. The 24-hour urine samples were centrifuged without any preservative. The sum of excreted TPM, defined as indole melanogens or nitrogen melanogens, was determined in the obtained supernatants [7, 8].

Analytical procedure. The sum of excreted TPM in 24-hour urine samples was determined by am modified Matous and Duchoń method [9]. 0.5 cm^3 of 2% water solution of sodium nitroprussiate (always freshly prepared) was added to 4.0 m^3 of the supernatant obtained after sample centrifugation. Then, following careful mixing of the solution, 2.0 cm^3 of 10% potassium hydroxide solution and 0.2 cm^3 of icy acetic acid were added. Absorbance was measured for 20 minutes by means of a spectrophotometer (Perhin Elmer) with reference to the so-called blind trial, at the wavelength of 620 nm.

The amount of indole melanogens (TPM) excreted with urine in a 24-hour collection was determined on the basis of a standardization curve (Fig. 2), for which the correlation coefficient r = 0.995 and statistical correlation significance t_α=67.8 were calculated. The high level of the parameter t_α=67.8, compared to the tabular value t_t=2.01, indicates a substantial significance of the spectrophotometric method used [10]. Furthermore, the coefficient of method variation was determined. For this purpose, 3.0 ug/cm^3 (1.7 mmol) of indole solution was added to 10 samples of the urine supernatant, each of 4 cm^3 volume. The procedures were then conducted according to the methods of TPM determination [9].

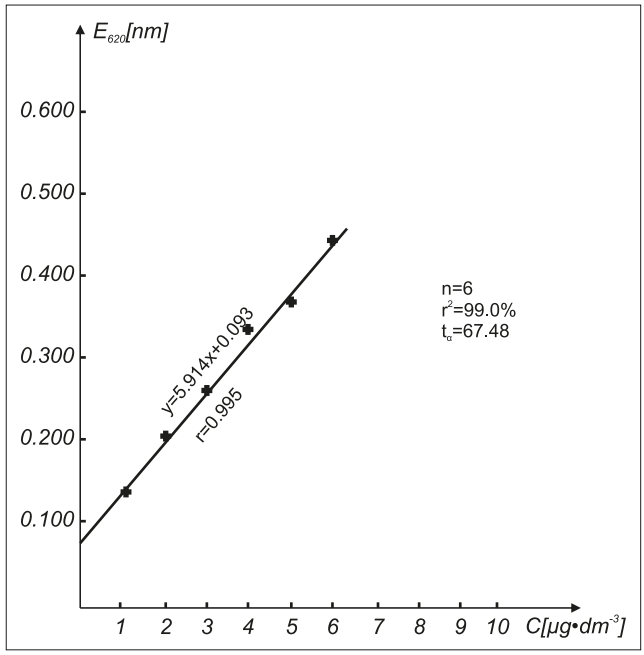

Figure 2. Calibration curve of indole concentration in 1 $dm^3$of urine

RESULTS AND DISCUSSION

The applied analytical method allowed quantitative assessment of the excreted indole melanogens (Thormahlen-positive) in correlation with the time of exposure of pigmented nevi to the action of solar radiation. The greatest TPM excretion was observed in August, when the study subjects were exposed to solar radiation for the longest time (180.8 hours) (Fig. 3). In all the cases, the level of excreted TPM increased, together with the exposure time, depending on individual characteristics. The changes in urinary TPM in relation to the exposure time are defined by the equation y = 0.0016 t + 0.37. The value of the absolute term b = 0.37 should be interpreted as an average TPM content, conditioned by prevalent environmental circumstances, such as arsenic concentration in suspended dust, water and food. At the same time, when the stimulating factor appears, i.e. solar radiation higher than average in the area, urinary TPM increases by 0.16 ug/dm^3 for each 100 hours of exposure. In the light of statistical analysis of the research results, the influence of the time of exposure to solar radiation is nature-dependent, which can be measured by the parameter a=0.0016. This can

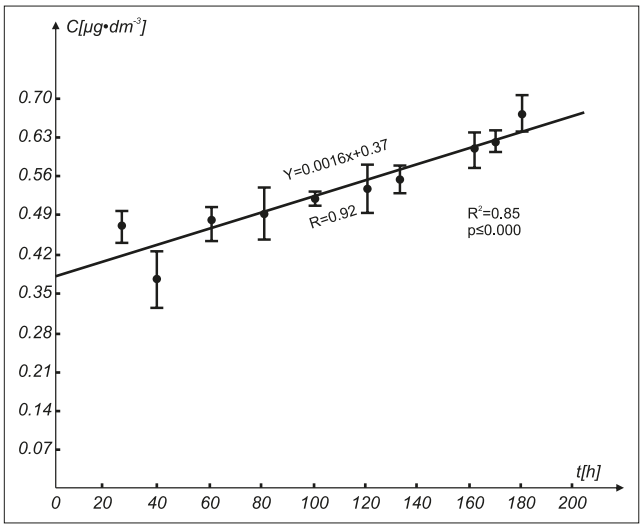

Figure 3. Urinary indole excretion (TPM) (C[µg/dm^3]) depending on time of exposure to solar radiation (t [h])

be explained according to the interpretation suggested by Pathak [11], who has proved [1] that the exposure to long-wave UV-A radiation is followed by enhanced processes of immediate pigment darkening (IPD) and initiation of additional melanogenesis. Indole melanogens arise from dopachinon, which is susceptible to nucleophilic addition. When thiols are absent, the dopachinon side-chain amino group acts as a nucleophil and the indole ring is formed. These indole substances are the basis of the Thormahlen test [12]. Some scientists suggest determination of the level of indole melanogens in fresh urine samples without stabilizing additives [9, 13]. The statistical picture of changes in urinary TPM content is presented in Table 1. The values are lower compared to the data reported by Matous and Duchoń [9] from former Czechoslovakia (3.23± 1.02 ug/dm^3). However, it is difficult to further compare the ranges of urinary TPM content with the findings provided by Matous, who did not take into consideration the time of exposure to solar radiation, either in the interpretation of findings or in observation scheduling. Changes in urinary TPM are presented in Fig. 4. The maximum TPM level (0.67 ug) was observed in August. The proportion of TPM content to the time of exposure in respective months should be noted. The obtained results allow the conclusion that the excretion of indole melanogens (TPM or nitric) is highly dependent on the time of exposure of pigmented nevi to solar radiation. This is indicated by high correlation and determination coefficients between the length of exposure of pigmented nevi to solar radiation and the level of excreted TPM. As the involvement of the ultraviolet spectrum part of solar radiation is higher in polluted regions, an increase in the incidence of skin tumours can be implicated, which will be the subject of further research.

The elevated urinary level of TPM is caused by the effect of solar radiation passing through the atmosphere at a greater angle of incidence. In spring and autumn, solar radiation is observed on a greater diagonal pathway through the layers of the atmosphere, and at a smaller angle of incidence, which is not without significance.

As urinary TPM levels are always region-specific, the study was based exclusively on the study population. It should be added that the reconstruction of the control group with similar behavioural features in the presented study field is rather unlikely.

The study population and the comparative group would be affected by solar radiation similar in spectrum and intensity of its lines. This is confirmed by the data presented in the Introduction, indicating that such measurements are of regional significance.

Table 1. Urinary content of TPM

Parameters		I	VI	VIII	XI
mean concentration µg/dm^3	4-10 years	0.3	0.35	0.46	0.2
	25-34 years	0.62	0.7	0.77	0.55
	35-45 years	0.5	0.63	0.76	0.41
	45-56 years	0.46	0.52	0.69	0.32
	whole group	**0.47**	**0.55**	**0.67**	**0.37**
standard deviation µg/dm^3		0,05	0.06	0.06	0.045
insolation (h)		26,8	133.0	180.8	39.6

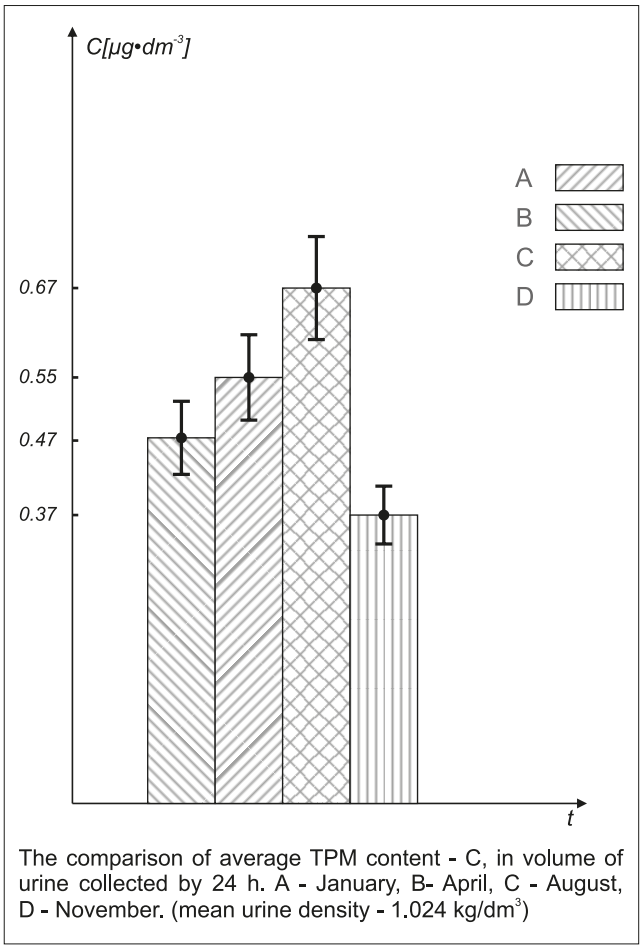

Figure 4. Average TPM content in the total 24-hour urine volume with reference to average urine density of 1.024 kg/dm^3

CONCLUSION

1. The excretion of indole melanogens (TPM) depends on the time of exposure to solar radiation, which in Upper Silesia amounts to 0.37 ug/dm^3 and increases by 0.16 µg/dm^3 in the urine for each 100 hours of the exposure.

2. The sunlight was found to stimulate melanogenesis in the study population. The maximum increase in the level of TPM was observed in August in the 25–45 age group (0.76–0.77 ug/dm^3), whereas the mean value for the study population was 0.67 ug/dm^3. The results have regional significance.

Acknowledgement

The study was supported by the Foundation 'Energetyka na rzecz południowej Polski' (Energetics for Southern Poland) and the Institute of Occupational Medicine and Environmental Health in Sosnowiec, Silesia, Poland.

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