

ANTIGENIC PROPERTIES OF UV IRRADIATED DNA AS A FUNCTION OF SPECTRAL REGION AND TIME

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It is already known that UV irradiation in systemic lupus erythematosus (SLE), a disease of unknown etiology, denatures DNA in cells of the skin. We considered the change of antigenic properties of UV irradiated DNA depending on UV spectral region and time of exposure to UV rays. Native DNA was irradiated and tested with antibodies from anti-DNA positive sera of lupus patients. We obtained negative DNA-anti DNA reactions ranging from 32,10 to 91,67 % of the control for the spectral region peaking at 254 nm, and from 12,50 to 41,66 % for the spectral region peaking at 365 nm. The data agree with the etiology of SLE but also indicate a specific type of denaturation caused by UV rays differing from thermal or sonar denaturation. The results also give support to further investigations in respect to the formation of antibodies characteristic for the time of UV irradiation.

Key words: DNA - anti DNA, UV irradiation, denaturation, systemic lupus erythematosus (SLE)

INTRODUCTION

The possibility of denaturation of DNA molecules exposed to ultraviolet (UV) light is known (Kripke, 1994, Vasilev *et al.* 2001). It has been shown that in the case of systemic lupus erythematosus (SLE), a disease of unknown etiology the irradiation with UV rays denatures DNA in cells of the skin (Dmitrieva *et al.* 2001, Paronich *et al.* 2001). This denaturation is followed by formation of pyrimidine dimmers of thymine or uracil (Jarzabek - Chorzeliska *et al.*, 1976) possibly responsible for the immunogenicity of UV-DNA molecules which cannot be repaired in SLE. Specific antibodies for UV-DNA and s-DNA (DNA denatured by means of sound) have already been described (Savic - Đurković *et al.*, 1985). Therefore, we divided our attention in to two routes: firstly to find out how the antigenic properties of UV-DNA molecules change with duration of UV irradiation, secondly, to compare different UV spectral regions and their effect upon antigenicity. For our first purpose we observed the formation of UV-DNA molecules as a function of time, while UV lamps emitting in different spectral regions were used, for the second.

MATERIALS AND METHODS

The experiment was performed *in vitro* (Tan, 1968.). Native DNA from salmon sperm was used as the antigen. Twenty four anti-DNA positive sera, taken from lupus patients were investigated. Anti-DNA positively was previously Crithidia tested (Arden, 1975). Native DNA samples from the salmon sperm unexposed to UV irradiation were used in control groups. The same serum was used for each analysis and control. The antigenic properties of DNA i.e. the presence of anti-DNA antibodies after UV irradiation of DNA molecules was detected by antigen-antibody interaction, using the method of "counter" electrophoresis (Kurata and Tan, 1976).

Two sources of UV light were used. The first (marked as lamp A) was a mercury lamp (Original Hanau type Hohenson HA-FI 3001), of 460W. It was shown on a spectrophotometer that this lamp emitted UV radiation peaking at 253.652 nm surpassing all other intensities. The second lamp (marked as lamp B) was a 100 W spot black light lamp (B-100 A, UV Products Inc. General Electric). It was a high pressure mercury arc lamp with light equally distributed in the visible and UV range. The maximum energy was obtained after filtering through a dark red-purple glass in the form of black light in the near UV region from 400 to 320 nm and peaked at 365 nm. The filter also removed all radiation below 300 nm but passed IR radiation of wavelengths longer than the visible red. The "spot" lamp concentrated its energy in a relatively small area over ≈ 15 cm diameter disc in a plane ≈ 38 cm from the black light filter. In the center of this circle the light intensity was about double the intensity at the edge of the circle.

Therefore, we chose a shallow round vessel of 8 cm diameter to contain the solution and irradiated it at a distance of 30 cm. The irradiation was always performed in a dark room and these conditions were kept the same for both lamps. When the aqueous solution of DNA (0.5 mg/ml) was poured into the vessel, it formed a layer 2 to 3 mm thick which was exposed to UV rays for different time intervals (5, 10, 15, 20, 25 and 30 minutes). As both lamps emit in the IR spectral region the temperature at the end of each time interval was measured, using a Pt-100 resistant digital Iskra thermometer with an accuracy 0,1 °C). The temperature was measured in air at a distance 30 cm from the source of irradiation with the ambient temperature signed as zero.

RESULTS AND DISCUSSION

Among the twenty four investigated anti DNA positive sera from lupus patients (taken as 100%) some of them did not interact any more with UV irradiated DNA molecules as antigens. The loss of antigenic properties varied depending on the duration of UV irradiation and type of Hg lamp used. It was shown that a longer time of irradiation always induced the formation of UV-DNA molecules more. Thus, we marked DNA -antiDNA reactions according to their negativity as a percentage of the control reaction (Table 1).

However, both lamps emit in the IR region, so the possibility of some thermal effect cannot be neglected. Therefore, temperature measurements characteristic for each time interval and for both lamps were made (Table 2). Longer periods of UV irradiation give rise to increased temperatures in the case of lamp A.

Table 1. Effect of UV irradiation upon the antigenic properties of DNA using anti - DNA antisera

Time of UV irradiation t (min)	DNA-anti DNA negative reaction %	
	Lamp A	Lamp B
5	32.10	12.50
10	45.83	16.66
15	69.64	25.00
20	75.00	20.16
25	83.34	33.33
30	91.67	41.66

Thus, the high percentage of native DNA-anti DNA reactions cannot be ascribed only to the influence of different spectral regions of Hg lamps. The results also agree with the finding that black light is not injurious and does not cause sunburn as UV light below 300 nm.

Table 2. Measurements of temperature as a function of time for the mercury lamps

Time t (min)	Temperature °C	
	Lamp A	Lamp B
0	22.0	23.2
5	38.8	24.5
10	40.4	25.3
15	43.7	26.7
20	45.3	27.4
25	46.4	27.6
30	47.6	28.0

In our previous work (Savić-Đurković *et al.* 1983.) we investigated thermal and sonar denaturation of DNA where purine and pyrimidine bases did not lose their function as antigenic determinants. UV denaturation induces reactions in the nucleic acid bases of DNA, such as photochemical oxidation, hydration and photodimerization where new photochemical radicals are formed (Levin *et al.* 1996, Shumiantseva *et al.* 2000, 1994). Adjacent pyrimidine residues on a DNA strand can become covalently linked if exposed to UV light forming dimers of thymine or uracil. The process of local denaturation, such as dimerization of thymine, is described together with formation of new cross-links stabilizing this kind of denaturation. UV irradiation obviously breaks antigenic determinants of DNA and its normal structure bringing about conformational alterations and changes in antigenic properties (Mil *et al.* 1974.) These changes affected the antigen- antibody reaction of DNA in our experiment. A definite type of conformation of DNA, different from the conformation of native DNA, has been found in lymphocytes of patients ill from SLE (Sano and Imokawa, 1983.) Anti - DNA antibodies can react both with native (double helical) and denatured (single helical) DNA or both. A

number of subtypes of anti-DNA antibodies reacting with single- helical DNA have been described (Kolke et al. 1982). Our results concern a specific type of denaturation caused by UV irradiation which differs from thermal and sonar denaturation in the immunological sense, too. There does exist the possibility that corresponding antibodies characteristic for UV-DNA are formed and that they depend on time of UV irradiation . Whether UV and thermal effects are cumulative or they bring about some qualitatively new changes in DNA molecules and their antigenic properties is the subject of our further investigation. The results obtained here are in accordance with aetiological findings for SLE already known in relation to sun exposure, so they may give support to some aspects in its pathogenesis.

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ANTIGENE OSOBINE UV ZRAČENE DNK U FUNKCIJI SPEKTRALNE REGIJE I VREMENA

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SADRŽAJ

Ultravioletni zraci (UV) indukuju pojavu sistemskog eritemskog lupusa (SEL) tako što denaturišu DNK u ćelijama kože. U radu je *in vitro* razmatrano ponašanje UV ozračenih molekula DNK kao antigena, kako bi se bolje proučio ovaj imunopatogenezni mehanizam. Nativna DNK sperme lososa *in vitro*, direktno je izlagana dejstvu UV zračenja određenih spektralnih regija, u različitom vremenskom periodu ozračivanja - od 5 do 30 minuta. Antigene osobine UV ozračene DNK (UV-DNK) ispitivane su *in vitro* preko reakcije DNK-anti DNK. Zračena DNK (UV-DNK) korišćena je kao antigen, a anti-DNK pozitivni serum bolesnika sa SLE kao antitela. Ultravioletno zračenje prouzrokovalo je promene, odnosno gubitak antigenih svojstava DNK. Zračena DNK (UV-DNK) nije mogla više da reaguje sa nekim anti-DNK pozitivnim, a istim serumima lupusnih bolesnika, sa kojima je inače reagovala pre ozračivanja. Procenat negativnih DNK - antiDNK reakcija sa UV-DNK kretao se od 32,10% do 91,67% za spektralnu regiju od 254 nm, a od 12,50 % do 41,66 %, za spektralnu regiju 365 nm pri vremenu ozračivanja od 5 do 30 minuta. Dobijeni rezultati pokazuju da promene antigenih osobina DNK zavise ne samo od upotrebljene određene spektralne regije UV zracena, nego i od vremena ozračivanja. Dobijeni rezultati ukazuju na specifičan vid denaturacije DNK pod dejstvom UV zracenja. On je različit u imunološkom smislu od termičke i ultrazvukom izazvane denaturacije, gde DNK zadržava sposobnost da kao antigen i dalje reaguje sa anti-DNK antitelima sa kojima je reagovala i pre denaturacije. Zračena DNK (UV-DNK) gubi ta imunološka svojstva da i dalje reaguje sa anti-DNK antitelima, sa kojima je inače reagovala pre ozračivanja. Dobijeni rezultati se slažu sa etiologijom SEL, ali ukazuju i na značaj daljih ispitivanja u odnosu na obrazovanje antitela karakterističnih za vreme UV - ozračivanja.