EFFECTS OF UVR ON IMMUNE RESPONSE OF SKIN AND EVALUATION OF SUNSCREEN

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Synopsis

1. It is an important work to monitor the ground level UVR, especially for a long time. The highest means UVR irradiate value at noon is observed in July (UV-420, 6814 µW/cm²; UV-365, 31 644 µW/cm²) or August (UV-297, 31.5 µW/cm²) at Shanghai. The trend showed that radiation power of summer > that of autumn > that of spring > that of winter. The highest means UVR of a day appeared around noon. The UVA is nearly 100 times higher than UVB.

2. Sun protection factor (SPF) is an important index to evaluate the effectiveness of sunscreen. It is urgent to establish the standards on evaluating the efficacy of sunscreen in our country. We have set up in vivo and in vitro methods to detect the SPF of sunscreen.

3. Though majority of people realized the harmful effects of UVR, they neglected to protect themselves from exposure to UVR. It is urgent to take some propaganda against UVR.

4. UVA radiation has the capacity to damage collagen I and collagen III. It is believed that UVA plays a important role in the induction of photodamage of the skin.

5. UVR can suppress immune responses of the skin. Cis-urocanic acid (cis-UCA) has been suggested as a photoreceptor for UV and has been demonstrated to suppress immune responses in delayed type hypersensitivity (DTH) in mice.

Riassunto

1. È un importante lavoro monitorare gli UVR a livello del suolo, soprattutto per uno lungo periodo di tempo. Il valore più alto di irradiazione degli UVR a mezzogiorno si rileva a luglio (UV-420, 6814 µW/cm²; UV-365, 31 644 µW/cm²) o agosto (UV-297, 31.5 µW/cm²) a Shanghai. La tendenza ha mostrato il potere di irradiazione dell'estate > di quello dell'autunno > di quello dell'inverno > di quello delle primavera. I più alti valori degli UVR in una giornata sono risultati essere quelli di mezzogiorno. Gli UVA sono circa 100 volte più alti degli UVB.

2. Il fattore di protezione dal sole (SPF=Sun Protection Factor) è un indice importante per valutare l'efficacia di uno schermo solare. È urgente stabilire gli standard per la valutazione degli schermi solari nel nostro paese. Noi abbiamo messo a punto metodi in vivo ed in vitro per rilevare il SPF degli schermi sol
3. Sebbene la maggior parte degli individui siano al corrente degli effetti nocivi degli UVR essi trascurino di proteggersi dall’esposizione agli UVR. È urgente iniziare delle campagne di informazione di massa sugli UVR.

4. Le radiazioni UVA hanno la capacità di danneggiare il collagene I ed il collagene III. Si ritiene che gli UVA giocino un ruolo importante nel fotodanneggiamento cutaneo.

5. Gli UVR possono annullare le risposte immunitarie della pelle. È stato indicato nell’acido cis-urocanico (cis-UCA) un fotorecettore per gli UV e è stato dimostrato che esso annulla le risposte immunitarie nella DTH (delayed type hypersensitivity) dei topi.
INTRODUCTION

Ultraviolet (UV) is one of the non-ionizing radiations in the electromagnetic spectrum and lies within the range of wavelength 100 nm to 400 nm. The short wavelength limit of the UV region is often taken as the boundary between the ionizing radiation spectrum (<100 nm) and the non-ionizing radiation spectrum. UV can be classified into UVA (315-400 nm), UVB (280-315 nm) and UVC (100-280 nm) regions, although other conventions for UVA, UVB and UVC wavelengths bands are in use.

Exposure to UV occurs from both natural and artificial sources. The sun is the principal source of exposure for most people. In some special situations, exposure may come from artificial sources which include various lamps used in medicine, industry, commerce, research and the home.

Short exposure to UVR may be beneficial for health as the exposed skin can generate vitamin D3. There are also deleterious effects on human health. The direct hazards are confined to the skin and the eyes because of the limited penetration of UVR into biological tissue. Excessive exposure can give rise to skin burns and blistering, which cause severe discomfort and systemic upset. Acute exposure may damage the cornea and conjunctiva, for example causing inflammation of the eye as the result of exposure to ambient UVR from surface such as snow with heightened reflectivity. Chronic exposure may also affect the skin by increasing aging effects and the risk of cancer and probably increases the risk of certain types of cataracts in the eyes. Small exposure to UVR can affect the skin's immune system and may enhance the risk of infection and decrease the effectiveness of vaccines in humans.

Globe environmental pollution becomes a more and more serious problem as industrialization being speeded up. With depletion of the stratospheric ozone layer, the environment will be exposure to higher intensities of UV. The sequences of this added UV exposure are considered so serious that it was a major topic for discussion at the World Environment Conference, held in Rio de Janeiro in 1992. In Agenda 21, adopted by the Conference, it was specifically recommended to "undertake, as a matter of urgency, research on the effects on human health of increasing ultraviolet radiation reaching the earth's surface as the consequence of depletion of the stratospheric ozone layer".

The increasing UV radiation will be a disaster to all living things including man. What shall we do? How can we prevent such unfortunate? As one of WHO Global Strategy for Health and Environment, a monograph has been drafted to provide the essential authoritative review on which future research programs in UV can progress. In recent years, researches on relationship between human and UVR have been taken into account in abroad. In contrast, there are few studies having carried out at home. As an environmental researcher, we should do our best to protect our living environment.

Part 1.

SOLAR RADIATION MEASUREMENTS

The sun is the main source of ultraviolet radiation (UVR). The stratospheric ozone layer, formed between 10 and 40 km from the surface, prevents almost all UVR of wavelengths less than 290 nm and a substantial proportion (70%-90%) of UVB radiation from reaching the earth. Recent public and scientific concern about ozone depletion and increased UV have lead to the establishment of many UV monitoring centers in the last few years. In 1980s less than 50 UV monitoring stations were operating around the world. Today more than 250 monitoring centers are underway for a variety of reasons (1). There are no systemic UV measurements reports in our China to date. In 1995, we start to measure ground-level solar radiation in Shanghai area.

MATERIALS AND METHODS

UV-A and UV-B radiometer (Light and Electric Instruments Factory of Beijing Normal School). UV
radiation was measured in sunny days, from AM 9.00 to PM 3.00, total three days in a month, one is among the first ten days of a month, another is among the middle ten days of a month, the third is among the last ten days of a month. There are no any reflective object or shadow around monitoring point.

RESULTS

The highest means UVR irradiate value at noon is observed in July (UV-420, 6814 µW/cm²; UV-365, 3184 µW/cm²) or August (UV-297, 31.5 µW/cm²) at shanghai. The trend showed that radiation power of summer > that of autumn > that of spring > that of winter. The highest means UVR of a day appeared around noon. The UVA is nearly 100 times higher than UVB.

DISCUSSION

It is an important work to monitor the ground level UVR, especially for a long time. It can be to provide information to the public on UV levels and variations and to establish a basic UV climatology (2). It can also study cause and effects of UV transmission and detect long term variability.

The World Meteorological Organization (WMO) has established a global network called Global Atmosphere Watch (WMO). It presently has eight observatory stations that make continuous spectral and broad band UV measurements.

The Global Environment Facility is supporting the creation of 10-15 additional stations in developing countries. Various national and multi-national agencies are also operating and establishing UV monitoring networks.

In our operation, we realize that only litter information can be obtained from manual monitoring, because data was limited by no continuous monitoring. It is very necessary to set up automatic monitoring station in our country.

Part 2.
A COMPARISON OF IN VIVO AND IN VITRO TESTING OF SUNSCREENS

Solar UVR is a main risk factor of skin aging and cancer. How to protect oneself from UVR become popular as awareness of UVR’s harm. In recent years, many kinds of sunscreens appeared in our country how to evaluate the effectiveness of sunscreen, sun protection factor (SPF) was usually used to evaluate the photoprotective efficacy. The SPF is defined as the ratio of the least amount of UVB energy (MED) required to produce a minimal erythema through a sunscreen product film to the amount of energy required to produce the same erythema without any sunscreen application.

Very little, has been done to evaluate the effectiveness of the commercially available sunscreens in our country. In this study, 8 commercial sunscreens and a 8% Homosalate standard substrate were compared on in vivo human, in vitro mouse skin and transpore surgical tape.

MATERIALS AND METHODS

1000 W xenon lamp (Light and Electric Instruments Factory of Shanghai) with a cut-off fliter WB 280/2mm (Colour Glass Factory of Shanghai) is used as solar simulator light source. UV-B radiometer (Light and Electric Instruments Factory of Beijing Normal School). Transpore surgical tape (Optometric Inc. US). SD mice were supplied by department of experimental animal, Shanghai medical university. Sunscreen was provided by cosmetic company.

Testing In vivo

Human sun protection factor (SPF) testing on a panel of 20 adult volunteers (6 females and 14 males) were conducted according to the Federal Register OTC Sunscreens Monograph of US (3). SPF is the ratio of Minimal Erytema Dose (MED) with sunscreens
to the MED without sunscreen. The skin type of 20 volunteers were type IV as are most of Chinese.

Testing *In vitro*
SPF was measured according Stockdale’s (4). Mouse skin was obtained from dorsal area of 5 day old SD mice. The epidermis was removed from dermal layer by immersing the mouse skin in 60 °C water for 30 seconds. The epidermal sheet of 2.5 cm in diameter were mounted on thin UVB transparent filter. Sunscreens were applied to mouse skin with 2 mg/cm². SPF was the ratio of the transmitting radiation through the untreated mouse epidermal sheet to that of the treated.

According to Furgson’s (5) method. Transpore surgical tape was used to test the SPF of sunscreens. The procedure of SPF measurement on the tape was just as on mouse skin.

**RESULTS**

The SPFs, based on human testing, of the sunscreens A, B, C, D, E, F, G, H were 2.16, 5.14, 6.85, 7.38, 8.31, 9.66, 3.5, 12.5 respectively.

There are no significant differences between the SPF detected with human *in vivo* and *in vitro* on transpore surgical tape. The coefficient of correlation is 0.9817 between human testing and transpore surgical tape. The SPF of 8% HMS sunscreen detecting with human and tape were compared with SPF suggested by FDA, there are no differences (U=1.37, 1.69; P>0.05).

There are no significant differences between the SPF detected with human *in vivo* and *in vitro* on mice skin except for G (t=2.33 P<0.05).

The coefficient of correlation is 0.9866 between human testing and mice skin. The SPF of 8% HMS sunscreen detecting with human and mice skin were compared with SPF (4.47±1.14) suggested by FDA, there are no differences (U=0.9556, 0.9556; P>0.05).

There are no significant differences between the SPF detected with tape and that with mice skin. The coefficient of correlation is 0.9860 between mice skin and transpore surgical tape.

**DISCUSSION**

Sunscreens had long history in western country, but only appeared in China in 1990s. A lots of consumer did not know much about the sunscreens, so it is very important to find a simple and valid method to evaluate the effectiveness of sunscreens and give advises to consumers.

In the present study, the 8 commercial sunscreens were tested by three different methods. The SPFs of these sunscreens ranged from 2.16 to 12.5.

Sunscreens with SPF 6 to 8 are suitable for Chinese people, according to the recommendation of FDA (6). *In vivo* human skin testing is the most essential and reliable method for determining SPF of sunscreens. But it takes a long time and makes photodamage to the tested skin of subjects.

It is very useful to detect the range of SPF of sunscreens before testing on human skin. *In vitro* mouse skin and transpore surgical tape were used to measure the SPF of 8 commercial sunscreens. The results showed a good correlation between the *in vivo* and *in vitro* methods. It indicated that it was possible to predict the SPF of sunscreens with *in vitro* testing. The testing with transpore surgical tape is more easier than that of mouse skin.

**Part 3. EPIDEMIOLOGICAL STUDIES OF SUN PROTECTION IN UNDERGRADUATES**

Sun exposure and sunburn, particularly in childhood, are important risk factors for skin cancer (7-9). In order to determine whether the youngsters are aware of the link between skin cancer and excessive exposure to sunlight and whether they know how to protect themselves from exposure to sunlight. Investigation were made to 368 undergraduates.
MATERIALS AND METHODS

Using a questionnaire, information was obtained about general conditions, history of sunburn, and diseases associated with sunlight, the awareness of UVR harmful effects and how to protect oneself. Sunscreen Testing: 40 female students, their skin type was IV, no light allergic or drug allergic history, are chosen from newcomers, A, B, C, D 4 groups were divided random with 10 people in each group. Sunscreen for UVB was used by group A, placebo was used by group B, sunscreen against UVB + UVA was used by group C, sunscreen for UVA was applied by group D, About 2mg/cm² of sunscreen was applied to the skin. A photo was taken before and after three weeks militarily exercise respectively. Using computerized imagine analysis techniques to measure the density of colour of 5 points in photo. The higher of density of color, the more pale of the skin.

RESULTS

There are 368 students involved in study, male and female are 50% each, averages of age is 18.6 years old, range from 16 to 21 years old, 5 (1.4%) had light allergic, 52 (14.1%) had sunburn, 2 (0.5%) had diseases associated with sunlight. 89.4% people are aware of UVR harmful effects. 70.4% consider that UVR can lead to skin aging, 88.2% agree with that UVR is risk factor of skin cancer. But only 13.6% take care of themselves away from UVR. 77.7% know about sunscreens, but only 17.7% understand the meaning of SPF. In military exercises, 12% of people applied sunscreens every day, 11.1% used sunscreens between times, 76.9% did not use anything. There were significant differences on density of color of photo taken before military exercise compared with that of after military exercise. It indicated that the skin color become darker after military exercise. There were no differences among A, B, C and D groups either before military exercise or after.

DISCUSSIONS

The investigation show that though majority of people realized the harmful effects of UVR, they neglected to protect themselves from exposure to UVR. The result was similar to that of investigation in western country (10-11). It is urgent to take some propaganda against UVR. It seems that the using of sunscreen in China is not popular as in western country. In human being testing of sunscreens, the results showed that the sunscreens did not supply any protection against UVR. The main causes of that may be sunscreen diluted by sweat, which is not rare during the military exercises. It indicated that waterproof sunscreens should be applied when doing some athletics. Another reason may be too many steps in processing the photo to distinguish the small differences. So it will be better to use computerized image analysis technique directly to detect the color of skin.

Part 4.

EFFECTS OF ULTRAVIOLET IRRADIATION ON HUMAN SKIN-DERIVED KERATINOCYTES AND FIBROBLAST IN VITRO

UVA radiation has the capacity to damage several cellular targets, including membranes, proteins and DNA, and the mechanism of such damage is believed to involve reactive oxygen species, which may have a variety of harmful effects, including the peroxidation of unsaturated lipids (12-13). It has been demonstrated previously that relatively large amounts of ultraviolet UVA can produce photodamage and it is believed that UVB plays a major role in the induction of photodamage and photocarcinogenesis. Recent, a study showed that even suberythemal doses of repetitive UVA may lead to photocaging of the skin. We studied the effects of UVA irradiation on human skin derived keratinocytes and fibroblasts by detecting a various antigen expressed by these two types of cells.
MATERIALS AND METHODS

CELL CULTURE AND UVA IRRADIATION. Keratinocytes and fibroblasts were cultured in 10 well slide. Seeding densities was 4x10^4 cells/ml, just as 1000 cells per well. The medium for keratinocytes was KGM, and for fibroblasts was RPMI, and for mixed was 66% KGM/34% RPMI. 25µl each cell suspension was put on to slides in square dish, put PBS at side to keep moist. Next day, check the density of cell, then add 10 ml medium, after three days, the cells were taken for irradiation. Before irradiation, the medium was aspirated and 5 ml HBSS were added to the dishes. Then put the dishes under the UVA lamp with the lid open and were irradiated to 10 mins, 30 mins, 60 mins, 90 mins and 120 mins respectively. Immediately after irradiation, the HBSS were aspirated and 10 medium were added and the cells incubated for another 24 hours. After 24 hours, the cells were checked for morphology and viability, then wash with PBS 10 mins for three times, and dry at room temperature, and stored in -70°C freezer.

IMMUNOHISTOCHEMISTRY. The cells reacted with primary antibodies P34 (1:50), GB3 (1:200), Plakoglobin (1:100), 11-5F (1:5), 3E1 (1:1000), G71 (1:100), Coll I (1:50), Coll III (1:50), Coll IV (1:50), Coll VII (1:10), Fibronection (1:100), FSP (1:200) for 60 minutes at 37°C. After 10 minutes wash for three times, the cells reacted with FITC labeled secondary antibodies for 30 minutes at 37°C. After 10 minutes wash for three times, the slide was mounted with citiflour. The slide was observed on olympus microscope by three individual. The density of fluorescence in cells was recorded as -, 1+, 2+, 3+, 4+.

RESULTS

The results showed that as the dose of UVA irradiation increase, the degree of staining in all cells become decrescent. Especially for Coll I and Coll III, when the dose of UVA were 3 J/cm², the staining faded, and the dose of UVA were 9 J/cm², the staining disappear.

DISCUSSION

Dermis contains predominantly type I collagen, with lesser amounts of type III collagen. The individual polypeptide chains of types I and III collagens are synthesized by dermal fibroblasts, as precursor molecules, procollagens, which contain globular amino and carboxy terminal domains. Within the cell, the individual chains assemble into trimeric type I or III procollagens, which are secreted into the extracellular space as soluble proteins. During formation of insoluble type I collagen fibrils, the carboxy and amino terminal domains are cleaved by specific protease, giving rise to pN collagen and pC collagen, respectively, which assemble into thin fibrils (14). Recent study showed that type I and type III collagen precursor levels are significantly reduced in severely photodamaged human skin (15-16). Our results demonstrate from cellular level that UVA was a risk factor of skin aging.

Part 5.
THE EFFECT OF UVR AND UROCANIC ACID ISOMERS ON DELAYED TYPE HYPERSENSITIVITY IN MICE

Irradiation with ultraviolet B suppresses some cell-mediated immune responses to a variety of antigens, including contact sensitizers (17-18). Following UV irradiation there is modulation of Langerhans cells markers and keratinocytes are induced to synthesize and secrete tumor necrosis factor-α (TNF-α) (19). Cis-urocanic acid (cis-UCA) has been suggested as a photoreceptor for UV and has been demonstrated to suppress immune responses in several experiment (20). In the present study the effects of UVR irradiation on delayed type hypersensitivity (DTH) in mice were compared with that of cis-UCA.
MATERIALS AND METHODS

Urocanic acid, Ovalbumin and DNFB (Sigma). UV Lamp (Light and Electric Instrument Factory of Shanghai). Antigens of DNP60VA were prepared according to Yano's (21) methods. SD mice were divided into A, B, C, D, E and F 6 groups with 10 in each group. A is control. B injected with trans-UCA (200 mg), C injected with cis-UCA (200 mg), D injected with cis-UCA (400 mg), E injected with cis-UCA (600 mg), F irradiated with UVR as a single dose of 5 kJ/m2.

The mice were tested for DTH to DNFB 7 days after sensitization. Ear thickness were measured before the mice were challenged by injecting 10 µl of antigen into each ear pinna. The ear thicknesses were again measure per mouse. Suppression of DTH was determined by the formula: % suppression = (1 - net increase of experimental mice/net increase of control mice) x 100.

RESULTS

The suppression of DTH in group of C, D and E were 47.3%, 52.5%, 56.0% respectively. There are dose-response relationship (r=0.9820, tř = 5.196, P<0.05). There are no significant differences between control A and B. There are significant differences between C, D, E, F and A (t=23.13, 20.25, 44.67, 19.47, P<0.001). There are significant differences between C, D, E, F and B (t=14.70, 15.99, 18.37, 7.70, P<0.01). There are significant differences between D, E and C (t=2.309, P<0.05; t=4.768, P<0.01). There are significant differences between D, E and F (t= 3.36, 5.399, P<0.01).

DISCUSSION

There are two subpopulations of T helper cells, designate Th1 and Th2 which appear to be differentially affected by UV exposure. These two populations are thought to regulate different sets of immune responses. Th1 cell produce IL-2 and γINF as well as other cytokines, promote DTH responses such CHS, provide help for certain antibody subtype responses including complement-fixing antibodies, activate macrophages, and may be particularly important for dealing with antigens expressed on cell surfaces, such as viral and tumor antigens (22). Th 2 cell produce a different array of cytokines including IL-4 and IL-5 which promotes antibody responses. UVR causes the release of mediators from the skin which alter the antigen presenting capability of Langerhans cells as well as antigen presenting cells at other sites, resulting in the development of suppressor T-cell. It may be that these suppressor T cells are Th2 cells. The net result is the failure to activate Th1 cells and suppression of DTH responses thought to play an important role in host defences against certain types of tumors and microbial infections. The immune suppression is antigen specific and is long lasting. In previous studies, using a single dose of urocanic acid, in present study, three doses were applied. The result show good relationship of dose-responses. The effects of UVR irradiation was similar to group C (cis-UCA 200 µg).
REFERENCES


Effects of UVR on immune response of skin and evaluation of sunscreen

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