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The prevention of the occurence of ultraviolet B (UVB) induced hypoxanthine guanidine phosphoribosyl transferase (HGPRT) mutant cells by several commercial sunscreens - An in vitro study

Noor Ikhtiyati, K.Etnawati, Y.Widodo W
Department of Dermatology, Faculty of Medicine, Gadjah Mada University, Yogyakarta

ABSTRACT

Noor Ikhtiyati, K. Etnawati, Y. Widodo Wirohadidjojo - *The prevention of the occurrence of ultraviolet B induced hypoxanthine guanidine phosphoribosyl transferase mutant cells by several commercial sunscreens - An in vitro study*

This study was aimed at comparing the effect of three different SPF (Sun Protecting Factors) of sunscreens in the prevention of UVB (Ultraviolet B) induced mutant fibroblast cells. The study was done using a simple experimental study design. Three commercially sunscreens were coated on the plate cover surface of fibroblast culture wells. Fibroblasts were isolated from ten young boy foreskins and subcultured in 3 to 5 passages. Mutagenic transformation was done by irradiation with 6.5 J/m2 Coerman Solarium CTL 3111 as a source of UVB. In addition there were two groups which were unprotected for control group and untreated with UVB for counting cumulative population doubling ratio (cpd). Three days after UVB irradiation, the culture, except for an unirradiated group, were incubated in HGPRT (hypoxanthine guanidine phosphoribosyl transferase) enriched medium, and fibroblast which survived in those medium considered as mutant cells. Cpd was calculated based on cell multiplication of the untreated group. The ratio of mutant cell population (*mcp*) is quantified by dividing the number of mutant cells with cpd. The difference of mean mcp between three subgroup of sunscreens and unprotected group was analyzed with Student's t test, and the difference of mean mcp among three subgroup of sunscreens was analyzed with ANOVA. The result showed that the mean mcp of sunscreen covered cultures were lower compared to the uncovered cultures and the higher SPF had lower mean of mcp significantly (P). Therefore sunscreen was proven to protect the induction of HGPRT mutant fibroblast although the protection was not totally, and the higher SPF sunscreen showed higher protection.

Key words : sunscreens - SPF - UVB - HGPRT gene mutation - fibroblast culture.

ABSTRAK

Noor Ikhtiyati, K. Etnawati, Y. Widodo Wirohadidjojo - *Pencegahan fibroblas mutan yang diinduksi oleh ultraviolet B pada berbagai tabir surya. Penelitian invitro.*

Penelitian ini bertujuan untuk membandingkan daya cegah beberapa macam tabirsurya yang memiliki SPF (Sun Protecting Factor atau Faktor Pelindung Surya) berbeda, terhadap timbulnya fibroblas mutan yang diinduksi oleh sinar ultraviolet B (UVB) secara in vitro. Penelitian dilakukan dengan desain eksperimental sederhana, yaitu 3 macam tabirsurya dengan SPF yang berbeda, dioleskan pada permukaan penutup lempeng sumuran biakan fibroblas. Fibroblas yang digunakan pada penelitian ini adalah fibroblas yang diisolasi dari 10 kulit preputium anak-anak, kemudian dilakukan subkultur 3-5 kali. Mutasi dilakukan dengan penyinaran spektrum Ultraviolet B yang berasal dari lampu Coerman solarium CTL 3111 dengan dosis 6,5 J/m2. Selain kelompok yang dilindungi oleh 3 macam tabirsurya tersebut, ditambahkan juga satu kelompok yang tidak dilindungi tabirsurya sebagai kontrol dan satu kelompok lagi yang tidak mendapat penyinaran untuk menghitung angka cpd *(cummulative population doubling ratio* - pengganda populasi sel kumulatif). Tiga hari setelah penyinaran, pada medium biakan fibroblas, kecuali yang tidak mendapat penyinaran, ditambahkan *hypoxanthine guanidine phosphoribosyl transferase* (HGPRT). Fibroblas yang masih tetap hidup pada media baru ini, dianggap sebagai fibroblas yang telah mengalami mutasi. Angka cpd dihitung berdasarkan jumlah sel hidup pada kelompok yang tidak mendapat penyinaran, dan angka populasi sel mutan atau mcp (*mutant cell population ratio*) dihitung dengan membagi jumlah sel mutan dengan cpd. Perbedaan rerata mcp antara ketiga kelompok tabir surya dengan kelompok kontrol dianalisis dengan menggunakan uji t, sedangkan perbedaan rerata mcp antara ketiga

macam tabir-surya dianalisis dengan menggunakan ANOVA. Hasil penelitian menunjukkan bahwa rerata mcp antara kelompok yang dilindungi oleh tabir surya dengan kelompok kontrol berbeda secara bermakna ($p<0,05$) dan semakin besar SPF, semakin kecil rerata mcp ($p<0,05$). Disimpulkan, meskipun perlindungannya tidak total, tabir surya yang diteliti terbukti dapat mencegah timbulnya fibroblas mutan yang diinduksi oleh sinar UVB dan semakin besar SPF semakin besar pula daya lindungnya.

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INTRODUCTION

To prevent human skin from harmful effect of solar radiation, sunscreen has been used for a long time and the potency of sunscreen especially in the ultraviolet-B (UVB) portion of the sun spectrum has been noticed as the sun protection factor (SPF)[1,2]. The SPF is a numeric notation to figure the comparison of minimal erythematous dose (MED) of solar radiation between sunscreen protected skin and unprotected one. In fact, MED is rather described the acute skin reaction against solar radiation than chronic one, therefore the SPF more figuring the potency of sunscreen against sunburn than chronic effect of solar radiation. The meaning of SPF for others harmful effect of solar radiation is still questionable.

Ultraviolet B (UVB) is the most potent ultraviolet light that reaches the earth surface. This spectra is capable not only to induce sunburn or skin pigmentation but also capable to stimulate skin cancer as well as skin aging process[3,4]. The UVB was known to react with pyrimidine base of DNA to produce cyclobutane pyrimidine dimer if uncorrected[5,6]. The persistent, uncorrected pyrimidine dimer, along DNA strand is well known as a basic of gene mutation. If this mutation affect genes responsible for cell replication, the initiation of tumorgenesis is then initiated[7]. Factually, the incidence of skin cancer has been increased and it has probably due to ozone depletion caused by the environmental change especially the industrial pollutants. The depletion of the ozone layer permits the solar energy in the earth surface increased especially the UVB spectra.

The mutation induced by UVB is occurred randomly along DNA strands but some genes are well known more susceptible and one of these susceptible genes is HGPRT (hypoxanthine guanidine phosphoribosyl transferase) gene. Mutation of HGPRT gene makes the cells cannot use the *de novo pathways* in their DNA synthesis. They have to use *the salvage pathway* in their DNA synthesis. Adding of external HGPRT will lead to the death of normal cells while the mutant one still viable[8,9]. This techniques of UVB induced HGPRT mutant cells is usually used in studying UVB induced mutant cell susceptibility, for example the UVB induce HGPRT mutant fibroblast occurs more frequent among fibroblast isolated from xeroderma pigmentosum patients than normal individuals[10,11].

The aim of this study is to compare the in vitro prevention of several SPFs suncreens against UVB induced HGPRT mutant fibroblast.

MATERIAL AND METHODS

Three to five passages of subculture fibroblasts, which were isolated from 10 foreskin healthy young boys, were used as donors for this study. These fibroblasts were cultured in Dulbecco's minimal essential medium (DMEM-ICN Flow, Meckenheim, Germany) with 10% fetal bovine serum (FBS-Seromed, Germany), L-glutamine 2mmol/l, penicilline-streptomycine-gentamycine, and fungizone with HEPES.

The UVB energy was achieved from Coerman solarium type CTL 3111 and the tested sunscreen were: Dermacos sun block lotion contain ethylhexylcinnamate 7.5%, benzophenone 6.0%, titanium dioxide 2.0% (SPF = 15); Special defense sunblock Clinique contain titanium dioxide 9.7% (SPF = 25); Sebamed Sunblock Cream contain octhyl methoxycinnamate 9% and titanium dioxide 7% (SPF= 28). For selection of mutant fibroblast, medium enriched with hypoxanthine guanidine phosphoribosyl transferase was used as a selection media.

A simple experimental study designed was used and subculture fibroblast were divided into three groups (Group I: consist of 3 subgroups (each was 10 subjects), each subgroups protected

with difference SPF sunscreens, Group II: unprotected one (10 subjects), and the non-UVB exposures was as group III or untreated group (10 subjects). Any group or subgroups had fibroblast from same subject and passage.

Isolation of fibroblast

Primary culture

The primary culture was taken from foreskin of circumcision of 10 healthy young boys and culture technique was done according to standard procedures[12,13]. In order to get fibroblasts as many as needed; the subculture should be done by trypsinisation of primary culture previously.

Fibroblast isolation

After subculture fibroblast had been grown confluent completely, media was aspirated; the rest of FBS (fetal bovine serum) then was cleaned with adding and taking out of PBS (phosphate buffered saline) twicely. After that, cells were removed with 0.02% EDTA (ethylendiamine tetraacetic acid)/ 0.25% trypsin for 3-5 minutes. The activity of trypsin was stopped by adding FBS containing media, and cells suspension were centrifuged for 5 minutes on 1000 rpm. Supernatant was taken out and the aggregate was dispensed into 1000 µl of the medium. Ten µl of the suspension was stained with 90 µl Turk's solution (consist of 1%, acid acetic. 0.01% in gentian violet in aquadest) and number of viable cells could be counted in the hemocytometric chambers under 100 times magnification of inverted microscope.

Subculture and treatment

Preparing of culture plate

All of the external side of wall microculture plates were covered with 80% darkness filmglass except for the outer part of cover plate directly above of the well column. The uncovered filmglass of the group were applicated with 2 mg per cm square sunscreen cream and the other groups were let be opened. For the prevention of solar scatter, all of the plates were kept in the dark container until treatment would be done. All procedures were done aseptically and resterilized with formallin.

Subculture and radiation

Three groups of subculture were done in DMEM contained 10% FBS started with 1ml of 10^5 per ml viable cell concentration for each. Every group had cells from the same passage. After 24 hours of cultivation, by using sterile micropipette, medium were aspirated and cleaned with PBS for twice. Group II and I were radiated with 6.5 J/m^2 of UVB (Coerman solarium type CTL 3111), and group III was refilled with medium. Immediately after radiation, the PBS was aspirated and refilled with culture media, and then all of subcultures were incubated in 37°C, 5% CO_2 for 72 hours.

Isolation of HGPRT mutant fibroblast

After 72 hours of incubation, the medium of both groups II and I was changed with medium enriched with 10 µl HGPRT per ml of medium and reincubated for 72 hours. The medium of group III was changed with medium without HGPRT. The medium of all groups were then aspirated, cleaned with PBS, treated with EDTA and trypsin, stopped with FBS. After centrifuging, supernatant (contained of killed cells) was taken out and the aggregate resuspended with 100 µl medium, and the viable cells could be counted according to the former procedures described.

Statistical analysis

Based on total viable cells of group III, cell population doubling ratio (cpd) is measured using as follow : $cpd = Ln\ N/\ 10^5 : (ln\ 2)$, whereas N was total viable cells from group III. The ratio of mutant cell population (mcp) is quantified by dividing of number of mutant cells with cpd

In order to see the effect of tested sunscreen in prevention of HGPRT mutant fibroblasts, the mean of mcp from Group I were compared against group II and tested by using Student's t test. The differences of mean mcp among tested sunscreens were tested with one way ANOVA.

RESULTS

The count population doubling of fibroblast (cpd) of untreated groups from any subject was as follows:

TABLE 1. - The number of UVB induced HGPRT mutant fibroblast based on treatment groups

Subjects	cpd (untreated)
1	0.79
2	1.37
3	0.96
4	1.17
5	0.26
6	1.37
7	1.41
8	1.62
9	0.94
10	1.49

This table shows that the range of cpd was 0.26 to 1.62. The comparison of mean mutant cell population (mcp) ratio between protected and unprotected group was as follows:

TABLE 2. - The comparisons of mean mcp between sunscreen protected groups and unprotected ones

Protected groups (n =10)	Unprotected group (n = 10)	statistical analysis
Sunscreen I:		t = 1.849;
71.445 ± 32.572	136.002 ± 105.490	P = 0.0405
Sunscreen II:		t = 2.345;
54.081 ± 32.770		P = 0.0153
Sunscreen III:		t = 3.018;
32.671 ± 24.398		P = 0.0037

All of the sunscreen protected groups significantly (P) showed lower mean mutant cell population ratio (mcp) compared to the unprotected group.

TABLE 3. - The Comparison of mean mcp among difference SPF

Sun Protection factor	Mean mcp	statistical analysis
SPF = 15	71.445 ± 32.572	
SPF = 25	54.081 ± 32.770	F = 4.145
SPF = 28	32.671 ± 24.398	P = 0.0269

It is shown in TABLE 3 that in the protected groups, the higher SPF of the sunsreens showed fewer mean mutant cell population ratio (mcp) ($p < 0.05$).

DISCUSSION

Spontaneous HGPRT mutation in fibroblast culture was 6 per 10^6 cells per-generation due to either cytosine deamination or genome error during DNA synthesis[14]. Because the persistent damaged DNA would be copied in S phase, the frequency of mutant cells depend on the opportunity of cells to repair their damaged DNA before synthesis DNA started. Destruction DNA in early S phase made the cells had no time to repair them. Based on some author's reports, the best time to induce mutation is in early S phase[15,16]. In the fibroblast culture, most of the cells entered the early S phase 24 hours after cultivation. This is the reason why UVB radiation in this study was done 24 hours after cultivation.

TABLE 1 shows that cpd of subjects differed from each other. The lowest cpd was 0.26 and the highest one was 1.62. This difference may occur due to the difference of the donor ages and difference of cell passage. Since the treated group and untreated one may come from the same subject and cell passage, the variation of cpd did not influence the predicted results.

TABLE 2 showed that, although total prevention was not found in any tested sunscreen, the mean of mcp between treated and untreated group differed significantly ($p < 0.05$). In other words, the using of sunscreen could protect the cell culture from UV induced mutant transformation. In this study, the parameter being used was mcp. Other authors used the difference parameter, mutant gene, and difference of amount of UV energy. Patton et al., using 6 thioguanin gene, 4 J/m^2 of UV energy, reported that the frequency of mutant cell was 50 cells per 10^6 normal individual fibroblast and 325 per 10^6 of xeroderma pigmentosa's fibroblasts[9]. Later, Watanabe et al. and Grossmann et al.[10,17] succeeded in increasing the frequency of mutant cells by improving the moment of UV exposure on cells culture.

TABLE 3 shows that the mean mcp was decreasing in parallel with the increasing of SPF significantly ($p<0.05$), or sunscreen with higher SPF had higher protection against UV induced mutation.

CONCLUSION

Each of the tested sunscreen was proven to protect the fibroblast from UVB induced mutation although the protection was not total. The higher SPF showed higher protection.

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