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

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ABSTRACT

Noor Ikhtiyari, K. Enawwad, Y. Wido W - The prevention of the occurrence of ultraviolet B (UVB) induced hypoxanthine guanine phosphoribosyl transferase (HPRT) mutant cells by several commercial sunscreens - An in vitro study

This study was aimed at evaluating the effect of three different SPF (Sun Protecting Factor) sunscreens in the prevention of UVB Ultraviolet B-induced mutant fibroblast cells. The study was done using a simple experimental study design. Three commercially-available sunscreens were coated on the plate cover surface of fibroblast culture wells. Fibroblasts were isolated from ten young boy forearm skin and subcultured in 3 to 5 passages. Ultraviolet radiation was done by irradiation with 6.5 J/m² Cwman Solarus CM 3111 as a source of UVB. The cell densities were adjusted to 5 x 10⁴ cells/well in 75 cm² flasks, and fibroblasts in these flasks were exposed to ultraviolet A and B 7.0 J/m² divided into two doses. The cell medium was replaced by culture medium containing 6.5 J/m² Cwman Solarus CM 3111 as a source of UVB, and fibroblasts in these flasks were considered as mutant cells. Cpd with different concentrations were added to the cell cultures, and the cell medium was replaced by culture medium containing 6.5 J/m² Cwman Solarus CM 3111 as a source of UVB. The results showed that the mean map of anucleate converted cultures was lower compared to the unmodified cultures and the higher SPF had lower mean of map significantly (P). Therefore sunscreen were proven to protect the induction of HPRT mutant the most although the protection was not totally, and the higher SPF sunscreen showed higher protection.

Key words: sunscreen - SPF - UVB - HPRT gene mutation - fibroblast culture.

ABSTRAK

Noor Ikhtiyari, K. Enawwad, Y. Wido W - Percepatan fibroblas mutan yang disebabkan oleh ultraviolet B pada berbagai tahap surya. Pemecatan invivo.

Penelitian ini bertujuan untuk membuktikan bahwa beberapa nasaam dalam SPF (Sun Protecting Factor) bisa menumbuhkan fibroblasts mutan yang disebabkan oleh sinar ultraviolet B (UVB) secara in vitro. Penelitian dilakukan dengan metode eksperimental sedemikian rupa, yaitu isolasi fibroblasts pada 3 medium selama 24 jam dengan SPF yang berbeda, dibuatkan pada permukaan gelas tampak dalam fluoresensi. Fibroblasts yang digunakan pada penelitian ini adalah fibroblasts yang dilakukan selama 10 kali pertambahan agar-agar, kemudian dilakukan penjilauan 3-5 kali. Metode dilakukan dengan proses penumbuhan fibroblasts dan diperoleh dari 6.5 J/m² Cwman Solarus CM 3111 dengan dose 6.5 J/m². Selain komponen yang dinilai sebanyak 200 kasus, dibentuk dengan keadaan yang diambil dari singkat, dan setelah kemunculan lagi yang tidak mempengaruhi kemampuan untuk menghasilkan angka SPF (Sun Protecting Factor) dengan akurasi 90-95%. Juga terdapat penelitian, pada awal penumbuhan fibroblasts, termasuk yang dilakukan pada metode penumbuhan, pembentukan hypoxanthine guanine phosphoribosyl transferase (HPRT). Fibroblasts yang masih tertutup pada waktu waktu ini, dibuatkan sel fibroblasts yaitu yang tidak mengalami perubahan, selapa komposisi sel mutan dan SPF (Sun Protecting Factor) transferase. Dihitung dengan metode jumlah sel mutan dengan pegangan SPF. Pengendalian rerata SPF antara kelompok hitam luar dengan kelompok kontrol dilakukan dengan menggunakan uji t, selanjutnya peng endalan rerata SPF antara kelompok...
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 reher 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 radia-
tion. The meaning of SPF for others harmful ef-
fact of solar radiation is still questionable.

Ultraviolet B (UVB) is the most potent ultra-
visible 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 tumorigenesis is then mutated\(^7,8\). Factually, the incidence of skin cancer has been increased and it has possibly 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 HPRT (hypoxanthine

guanine phosphoribosyl transferase) gene. Mut-
ation of HPRT 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 HPRT will lead to the death of normal cells while the mutant one still viable\(^9\). This technique of UVB induced HPRT mutant cells is usually used in studying UVB induced mutant cell susceptibility, for example the UVB induce HPRT mutant fibroblast occurs more frequent among fibroblast isolated from xeroderma pigmentation patients than normal individuals\(^10,11\).

The aim of this study is to compare the in vitro prevention of several SPF sunscreens against UVB induced HPRT mutant fibroblast.

MATERIAL AND METHODS

Three to five passages of subculture fib-
broblasts, which were isolated from 10 fore\'skin healthy young boys, were used as donors for this study. These fibroblasts were cultured in Dul-
becco’s minimal essential medium (DMEM-ICN
Flow, Meckenheim, Germany) with 10% fetal bo-
vine serum (FBS-Senomed, Germany), L-glut-
tamine 2mmol/l, penicilline-streptomycine-gen-
tamycine, and fungaze with HEPEX.

The UVB energy was achieved from Coenan
solarium type CTL 3111 and the tested sunscreen were: Dermacon sun block lotion contain etty-
hexylcinamate 7.5%, benzophenone 6.0%, tita-
nium dioxide 2.0% (SPF = 15); Special defense sunblock Clinic contain titanium dioxide 9.7% (SPF = 25); Sebarred Sunblock Cream contain octyl methoxyphenylaminate 9% and titanium diox-
ide 7% (SPF = 28). For selection of mutant fi-
broblast, medium enriched with hypoxanthine

guanine phosphoribosyltransferase was used as a selective media.

A simple experimental study designed was used and subculture fibroblast were divided into three groups (Group 1: consist of 3 subgroups (each was 10 subjects), each subgroup protected
with difference SPF sunscreens, Group II: unpro-
ected one (10 subjects), and the non-UVB expo-
sures 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. In order to get fibroblasts as
masy as needed; the subculture should be done
by tarsminisation of primary culture previously.

Fibroblast isolation

After subculture fibroblast had been grown
confluent completely, media was aspirated; the
rest of PBS (fase bovine serum) then was cleaned
with adding and taking out of PBS (phosphate
buffered saline) twice. After that, cells were re-
moved with 0.02% EDTA (ethylenediamine
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. Super-
natant was taken out and the aggregate was dis-
pensed into 1000 µl of the medium. Ten µl of
the suspension was stained with 90 µl Turk’s solution
(consist of 1%, acid acetis. 0.01% in gentian vio-
et in aquadest) and number of viable cells could
be counted in the hemocytometric chambers un-
der 100 times magnification of inverted micro-
scope.

Subculture and treatment

Preparing of culture plate

All of the external side of well 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 applied 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 resterilizes
with formalin.

Subculture and radiation

Three groups of subculture were done in
DMEM contained 10% FBS started with 1ml of
10^7 per ml viable cell concentration for each.
Every group had cells from the same passage. Af-
ter 24 hours of cultivation, by using sterile mi-
cropette, medium were aspirated and cleansed
with PBS for twice. Group II and I were radiated
with 6.5 J/m^2 of UVB (Coerman solarium type
CTI 3111), and group III was refilled with me-
dium. Immediately after radiation, the PBS was
aspirated and refilled with culture media, and
then all of subcultures were incubated in 37°C,
5% CO2 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 radium without
HGPRT. The medium of all groups went then as-
pirated, cleaned with PBS, treated with EDTA and
tryptin, stopped with FBS. After centrifug-
ing, supernatant (containd 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 Nf/ Ln N0 (ln 2), whereas N f
was total viable cells from group III. The ratio of
mutant cell population (mcp) is quantified by di-
viding 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
(cpdl of untreated groups from any subject was as
follows:
### TABLE 1. - The number of UVB induced HGPRT mutant fibroblast lines on treatment groups

<table>
<thead>
<tr>
<th>Subjects</th>
<th>cpl (unrestr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.75</td>
</tr>
<tr>
<td>2</td>
<td>1.17</td>
</tr>
<tr>
<td>3</td>
<td>0.96</td>
</tr>
<tr>
<td>4</td>
<td>1.17</td>
</tr>
<tr>
<td>5</td>
<td>0.26</td>
</tr>
<tr>
<td>6</td>
<td>1.37</td>
</tr>
<tr>
<td>7</td>
<td>1.41</td>
</tr>
<tr>
<td>8</td>
<td>1.62</td>
</tr>
<tr>
<td>9</td>
<td>0.94</td>
</tr>
<tr>
<td>10</td>
<td>1.49</td>
</tr>
</tbody>
</table>

This table shows that the range of cpl was 0.20 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 sunscreens protected groups and unprotected ones

<table>
<thead>
<tr>
<th>Protected group (n=10)</th>
<th>Unprotected group (n=10)</th>
<th>statistical Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sunscreen I:</td>
<td></td>
<td>t = 1.840;</td>
</tr>
<tr>
<td>71.445 ± 32.572</td>
<td>136.00 ± 105.490</td>
<td>P = 0.04011</td>
</tr>
<tr>
<td>Sunscreen II:</td>
<td></td>
<td>t = 2.345;</td>
</tr>
<tr>
<td>54.081 ± 32.770</td>
<td></td>
<td>P = 0.00013</td>
</tr>
<tr>
<td>Sunscreen III:</td>
<td></td>
<td>t = 3.018;</td>
</tr>
<tr>
<td>32.671 ± 24.398</td>
<td></td>
<td>P = 0.0027</td>
</tr>
</tbody>
</table>

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 different SPF

<table>
<thead>
<tr>
<th>Sun Protection Factor</th>
<th>Mean mcp</th>
<th>statistical analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>SPF = 15</td>
<td>71.445 ± 32.272</td>
<td>F = 4.145</td>
</tr>
<tr>
<td>SPF = 25</td>
<td>54.081 ± 32.776</td>
<td>P = 0.029</td>
</tr>
<tr>
<td>SPF = 28</td>
<td>32.671 ± 24.398</td>
<td>P = 0.029</td>
</tr>
</tbody>
</table>

If it is shown in TABLE 3 that in the protected groups, the higher SPF of the sunscreens showed lower mean mutant cell population ratio (mcp) (p < 0.05).

### DISCUSSION

Spontaneous HGPRT mutation in fibroblast culture was 6 per 10⁸ cells per-generation due to either cytosine deamination or genome error during DNA synthesis. 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 is early S phase made the cells had no time to repair them. Based on some author’s report, the best time to induce mutation is in early S phase. 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 cpl of subjects differed from each other. The lowest cpl 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 cpl did not influence the predicted results.

TABLE 2 showed that, although total prevention was not found in any tested sunscreens, 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 gen, 4 J/m² of UV energy, reported that the frequency of mutant cell was 50 cells per 10⁸ normal individual fibroblast and 325 per 10⁸ xeroderma pigmentosum’s fibroblasts. Later, Watanabe et al. and Grossmann et al. succeeded in increasing the frequency of mutant cells by improving the amount 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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