The effect of topically extravirgin olive oil on the UVB-induced immunosuppression

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ABSTRACT

Yohanes Widodo Wirohadidjojo, Arief Budiyanto, Irianiwati- The effect of topically extravirgin olive oil on the UVB-induced immunosuppression.

Background: UVB radiation may act as an immunosuppressive agent through Langerhans cells (LCs) depletion correlated with cyclobutane pyrimidine dimer (CPD), as the most mutagenic photoproducts. Other studies showed that olive oil can prevent various human cancers, which are defect of immune-surveillance. The effect of olive oil in the UVB-induced LCs depletion is still unclear.

Objective: To discover the topical effect of extravirgin-olive-oil in the LCs depletion.

Methods: A simple experimental study was performed on foreskins collected from 8 circumcised boys. Each of them was cut into 3 pieces of 0.5 cm2, grouped randomly to: (1) olive oil + UVB group, (2) placebo + UVB group, and unirradiated group. They were cultured in DMEM for 24 hours, irradiated with 100mJ/cm2 UVB, and olive oil or placebo were then applied immediately after irradiation. All specimens were fixed in buffered formalin, paraffin blocked, and cut to be 2 slides, and each of them was stained with anti-CD1a or anti-CD2 antibody. The pixels of AEC signals were digitally counted based on Photoshop’s selected color histogram. The pixel means of various groups were analyzed by Kruskal-Wallis test and post hoc analysis by Mann Whitney test.

Results: Olive oil treated groups showed a very significant (P<0.01) higher level of CD 1a and lower level in CPD compared to UVB group.

Conclusion: Olive oil has a protective effect in UVB-induced LCs depletion through preventing the CPD formation.

Key words: UVB - CD1a - CPD - Olive oil - Human skin

ABSTRAK

Yohanes Widodo Wirohadidjojo, Arief Budiyanto, Irianiwati- Khasiat minyak zaitun topikal pada supresi imun akibat pajaran siner ultraviolet B.

Latar belakang: Pajanan UVB dapat berafek imunosupresif melalui depleksi sel Langerhans (SL) akibat pembentukan dimer pirimidin siklobutan (DPS) yang sangat mutagenik. Fakta menunjukkan bahwa minyak zaitun dapat mencegah bermacam-macam kanker yang dipangaruh oleh cecat imumnosurvailans. Efek minyak zaitun pada depleksi SL oleh UVB belum diketahui.

Tujuan: Mengetahui efek minyak zaitun topikal pada pencegahan depleksi SL karena UVB.

Metode: Eksperimen sederhana in vitro dilakukan pada explant kulit kulup yang dikumpulkan dari 8 anak lalaki yang disuntik. Kulit dipotong menjadi 3 potong berukuran 0,5 cm2, dicek menjadi kelompok: placebo, placebo + UVB, dan minyak zaitun + UVB. Penyinaran UVB diberikan dalam dosis 100 mJ/cm2, dan placebo atau minyak zaitun diolehkan segera setelah penyinaran. Setelah dinkubasi selama 24 jam dalam DMEM lengkap, kulit difiksasi, dibuat blok paraffin, dipotong menjadi 2 slide dan dipulas secara imunohistokimia dengan anti-CD1a dan anti-DPS. Pixel warna AEC digunakan untuk analisis bijak dengan komputer berdasarkan analisis histogram program Photoshop. Perbedaan ekspresi CD1a dan DPS antara kelompok diuji dengan analisis Kruskal-Wallis dan uji post hoc dengan uji Mann-Whitney.

Hasil: Dibandingkan dengan kelompok UVB, kelompok minyak zaitun menunjukkan peningkatan ekspresi CD1a dan penurunan ekspresi DPS secara sangat bermakna (P<0.01).

Simpulan: Minyak zaitun topikal terbukti dapat menghambat depleksi SL oleh siner UVB melalui hambatan pembentukan DPS.
INTRODUCTION

The researchers believe that UVB irradiation on human skin may play as photoimmuno-suppressant, since several studies have shown that contact sensitivity against DNCB as well as graft rejection among mice have shown inhibition by UVB radiation experimentally. This inhibition might due to the depletion of Langerhans cells (LCs), based on experimental study that UVB could prevent maturation and induced apoptosis of LCs, and the number of LCs of UVB irradiated CH3 mice were significantly decreased compared either with unirradiated or UVAl-irradiated mice.

Absorbing UVB photon by pyrimidine bases of a single strand DNA may stimulate a reaction with adjacent pyrimidine base to perform a cyclobutyl pyrimidine dimer (CPD). Now, researchers believe that CPDs are the most mutagenic DNA damages. Based on several researches, the failure of CPD repair may responsible in the occurrence of several gene point mutations, such as p53 gene and ras genes, which are known as the target initiation of carcinogenesis. CPDs were suggested responsible for LCs depletion, since the location of epidermal CPDs were the same with the location of epidermal LCs, treatment of UVB irradiated mice topically with liposome containing CPD repair enzymes could restore mice contact hypersensitivity, repaired CPDs were followed by restoration of LC’s ICAM-1 expression.

Olive oil is a vegetable oil which is largely consumed by Mediterranean people, it has an important role in the breast cancer prevention, and consuming this oil may prevent human colon cancer. Based on the purification technique, olive oil can be considered as: crude olive oil, virgin olive oil, and extravirgin olive oil. Application of extravirgin olive oil could prevent UVB induced DNA damage as well as UVB induced skin cancers. Olive oil is considered stable in water-oil emulsions and can be used in cosmetic preparation. The effect of olive oil in the prevention of LCs depletion is still unclear.

METHODS

A simple experimental study was performed on eight foreskins collected from circumcised 11 to 13 year old boys. Each foreskin was cut into 3 pieces of 0.5 cm² and they were divided randomly into UVB + placebo group, UVB + Olive oil group, and non-UVB group. They were skin explant cultured in DMEM completed medium (DMEM + Bovine serum 10% + Penicillin 100 IU/ml + Streptomycin 100 unit/ml + Amphotericin B 2,5 µg/ml) with air interface of epidermal sides in 0.5 µM filter surrounded by metal rings. After 24 hours, UVB radiations were performed at 100-mJs/cm², and 100uLs extravirgin olive oil as well as normal saline and placebo was applied immediately after irradiation. Tissues fixation, paraffin blocking, and cutting were performed at 24 hours later.

Two slides of each paraffin block were made, and each of them was stained with anti-CD1a, or anti-CPD and IHC Kit. Counter staining was performed with Hematoxyllin Mayer.

Histological images were captured digitally using 8 megapixels camera connected to ocular lens of 400 magnification light microscope (Olympus) in quadruplets for each histological slide.

Statistical analysis

CD 1a and CPDs expression were calculated based on the proportion of number pixel of positive cell/negative cells. Number pixels were determined based on the histogram of selected color of Photoshop computerized programme, as previously performed by Lehr et al. and considered as a good method in computerized quantification of antigenic colored histological expression. Abnormal distributions of data were analyzed by using Kruskall-Wallis test and post-hoc test with Mann-Whitney test.

RESULTS

All of experimental results can be observed in the following figures.
Based on this figure, 100 mL/cm² UVB was strong enough to deplete LCs very significantly (P < 0.01).

It can be observed that olive oil could prevent LCs depletion very significantly (P < 0.01).

Based on this figure, olive oil could prevent UVB-induced CPD formation significantly (P < 0.05).
DISCUSSION

FIGURE 1 shows that 100 ml/cm² of UVB was capable to stimulate epidermal LCs depletion as shown by a significant decrease in CD1a expression (P<0.01). This finding was not only an evidence of the correctly-performed protocol of UVB irradiation in this study, but also as a supporting finding of various previous studies such as: the effect of UVB on the LCs cytoskeleton, the effect of UVB on the number of epidermal LCs of mice, and the effect of UVB on local suppression of contact hypersensitivity among mice, since epidermal LCs had the most important antigen presenting cells in contact hypersensitivity.

Based on FIGURE 2, it could be concluded that topical olive oil had a protective effect in the UVB-induced LCs depletion as shown by a very significant higher CD1a expressions among olive oil treated groups (P<0.01). Olive oil was epidemiologically proven in preventing various human malignancy such as: breast cancer, colonic cancer, or DNA damages responsible in the oncogenes activation, now it has been proven in the prevention of UVB-induced Langerhans cells depletion, or by other words, it was capable to prevent UVB-induced surveillance immune suppressant as another basic mechanism of photocarcinogenesis. This mechanism may be based on either the capability of olive oil as an antioxidant or olive oil capability in prevention of the direct DNA damage, and is not due to olive oil as a light reflectance, since previous study showed that application of this material before UVB irradiation had no effect on oxidation as well as direct DNA damages formation. The CD1a expression among various experimental groups can be observed on FIGURE 3.

In FIGURE 4, it is showed that 100 ml/cm² of UVB was strong enough to stimulate CPD formation on human skin and application of olive oil immediately after UVB irradiation was capable to significantly reduce CPD formation in 24 hours later (P<0.01-FIGURE 5). This finding was slightly different with Budiyanto et al. reports. They found that application of olive oil, immediately after UVB irradiation on hairless mice significantly reduced oxidative DNA damages, but not significantly reduced direct DNA damages such as CPDs. The different skin thickness which may influence in the olive oil-non antioxidants ingredient absorption rates between hairless skin and human skin may be a cause of these differences. In addition, the CPD expression in various experimental groups can be observed on the FIGURE 5.

CPD formation may be responsible in the UVB-induced LCs depletion as postulated by some authors. Study on mice showed that both XPA mice with defective global genomic repair and CSB mice with defective transcribed genomic repair were much more sensitive for UVB-induced immunosuppression compared with XPC mice and wild-types littermates. Twenty to thirty percent of LCs in UVB-induced blister fluid of 30 healthy volunteers were positive for CPD, which coincided with reduced number of LCs in the blister roof. Based on those finding and supported by coincident reduction in CPD expression and increased CD1a expression in olive oil treated group leads to a conclusion that olive oil prevented LCs depletion may be based on the prevention of UVB-induced CPD formations.

UVB-induced LCs depletion can be inhibited by chemical sunscreen in human skin explants study. Since olive oil has been considered stable in water-oil emulsions and could be used in cosmetic preparation. Adding olive oil in various mixed sunscreens preparation may be a beneficial way in the prevention of photo-carcinogenesis, especially in people who were prone to UVB-induced skin cancers, such as xeroderma pigmentosum patients, but its effectiveness was still needed to be clarified clinically.

CONCLUSION

Extravirgin olive oil was proven to be capable to prevent UVB-induced LCs depletion and its mechanism might based on the prevention of UVB-induced CPDs formation. The effectiveness of sunscreen containing olive oil in the UVB-induced immunosupressant should be clarified.

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