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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 cyclobuthane 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 cm^2, grouped randomly to: (Olive oil + UVB)group, (placebo + UVB)group, and unirradiated group. They were cultured in DMEM for 24 hours, irradiated with 100mJs/cm^2 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-CPD 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 pajanan sinar ultraviolet B.***

Latar belakang: Pajanan UVB dapat bersifat imunosupresif melalui deplesi sel Langerhans (SL) akibat pembentukan dimer pirimidin siklobutan (DPS) yang sangat mutagenik. Fakta menunjukkan bahwa minyak zaitun dapat mencegah bermacam-macam kanker yang dipengaruhi oleh cacat immunosurveilans. Efek minyak zaitun pada deplesi SL oleh UVB belum diketahui.
Tujuan: Mengetahui efek minyak zaitun topikal pada pencegahan deplesi SL karena UVB.
Metoda: Eksperimen sederhana *in vitro* dilakukan pada *explant* kulit kulup yang dikumpulkan dari 8 anak lelaki yang disunat. Kulit dipotong menjadi 3 potong berukuran 0,5 cm^2, diacak menjadi kelompok: plasebo, plasebo + UVB, dan minyak zaitun + UVB. Penyinaran UVB diberikan dalam dosis 100 mJ/cm2, dan plasebo atau minyak zaitun dioleskan segera setelah penyinaran. Setelah diinkubasi selama 24 jam dalam DMEM lengkap, kulit difiksasi, dibuat blok paraffin, dipotong menjadi 2 *slide* dan dipulas secara imunohistokimiawi dengan anti-CD1a dan anti-DPS. Pixel warna AEC citra digital dihitung dengan komputer berdasar 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 deplesi SL oleh sinar UVB melalui hambatan pembentukan DPS.

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INTRODUCTION

The researchers believe that UVB irradiation on human skin may play as photoimmunosuppressant, since several studies have shown that contact sensitivity against DNCB as well as graft rejection among mice have shown inhibition by UVB radiation experimentally.[1] 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[2], and the number of LCs of UVB irradiated CH3 mice were significantly decreased compared either with unirradiated or UVAI-irradiated mice.[3]

Absorbing UVB photon by pyrimidine bases of a single strand DNA may stimulate a reaction with adjacent pyrimidine base to perform a cycloadduct, called 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[4,5] and ras genes[6], which are known as the target initiation of carcinogenesis.[7,8] CPDs were suggested responsible for LCs depletion, since the location of epidermal CPDs were the same with the location of epidermal LCs[9], treatment of UVB irradiated mice topically with liposome containing CPD repair enzymes could restore mice contact hypersensitivity[10], repaired CPDs were followed by restoration of LC's ICAM-1 expression.[11]

Olive oil is a vegetable oil which is largely consumed by Mediterranean people, it has an important role in the breast cancer prevention[12], and consuming this oil may prevent human colonic cancer.[13] 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.[14,15] Olive oil is considered stable in water-oil emulsions and can be used in cosmetic preparation.[16] 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 cm^2, 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 ug/ml + Streptomycine 100 unit/ml + Amphotericine B 2,5 ug/ml) with air interface of epidermal sides in 0.5 uM 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.*[17] 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.

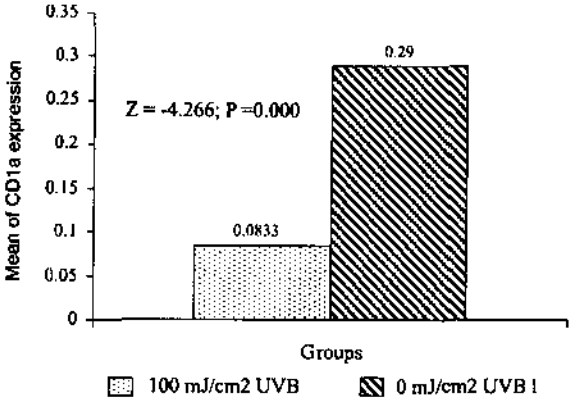

FIGURE 1. CD1a expressions between UVB and non-UVB treated skins

Based on this figure, 100 mJ/cm2 UVB was strong enough to deplete LCs very significantly ($P < 0.01$)

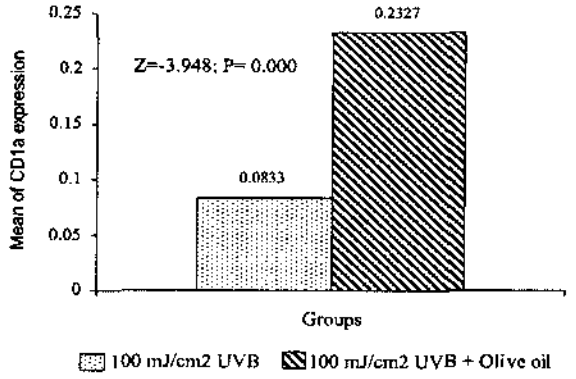

FIGURE 2. CD1a expressions between UVB and UVB + Olive oil treated skins

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

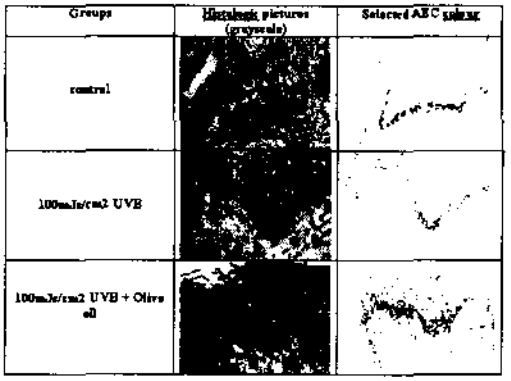

FIGURE 3. CD1a expression in various groups and their selected pixels. Olive oil treated groups showed more prominence in CDIa expression

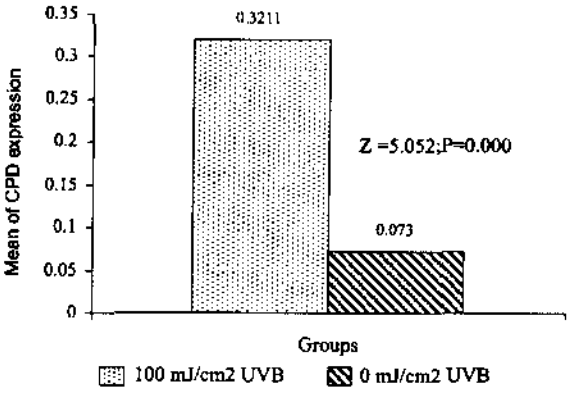

FIGURE 4. CPDs expressions between UVB and non-UVB treated skins

It shows that 100 mJ/cm2 UVB was capable to stimulate CPD formation very significantly ($P< 0.01$).

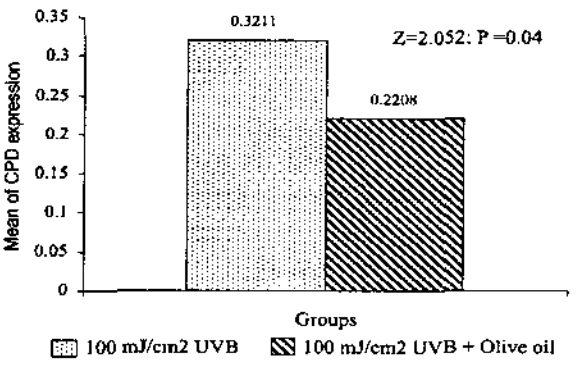

FIGURE 5. CPDs expressions between olive oil and placebo of UVB treated skins

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

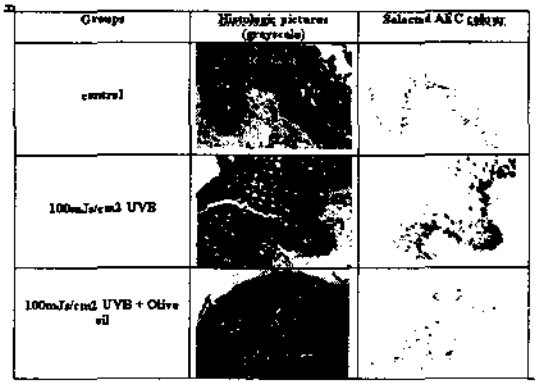

FIGURE 6. CPD expression among various groups and their selected pixels. Shows oil treated groups have a letter CPD expression

DISCUSSION

FIGURE 1 shows that 100 mJs/cm^2 of UVB was capable to stimulate epidermal LCs depletion as shown by a very 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[18], the effect of UVB on the number of epidermal LCs of mice[3], and the effect of UVB on local suppression of contact hypersensitivity among mice[10], 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[12], colonic cancer[13], or DNA damages responsible in the oncogenes activation[14,15], 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 photo-carcinogenesis. 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.[14] The CD1a expression among various experimental groups can be observed on FIGURE 3.

In FIGURE 4, it is showed that 100 mJ/cm^2 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[14]. 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.[9,10,11] 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.[19] 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.[20] 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.[21,22] Since olive oil has been considered stable in water-oil emulsions and could be used in cosmetic preparation.[16] 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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REFFERENCES

1. Bergstresser PR, Toews GB, Streilein JW. Natural and pertubed distributions of Langerhans cells: responses to ultraviolet light, heterotrophic skin grafting, and dinitroflourobenzene sensitization. J Invest Dermatol 1980;75:73-77.
2. Nakagawa S, Koomen CW, Bos JD, Teunissen MBM. Differential Modulation of Human Epidermal Langerhans Cell Maturation by Ultraviolet B Radiation. J Immunol 1999;163:5192-200.
3. Duthie MS, Kimber I, Dearman RJ, Norval M. Differential effects of UVA1 and UVB radiation on Langerhans cell migration in mice. J Photochem Photobiol 2000;57(2-3):123-31.
4. Drouin R and Therrien JP., UVB induced cyclobutane pyrimidine dimer frequency correlates with skin cancer mutational hot spot in p 53. J Photochem Photobiol 1997;66(5):719-26.
5. Hyong You Y, Szabo PE, Pfeifer GP. Cyclobutane pyrimidine dimers form preferentially at the major p53 mutational hot spot in UVB-induced mouse skin tumors. Carcinogenesis 2000;21(11):2113-17.
6. Kamiya H, Murata N, Murata T, Iwai S, Matsukage A, Masutani C, Hanaoka F, Ohtsuka E. Cyclobutane thymine dimers in a ras proto-oncogene hot spot activate the gene by point mutation. Nucleic Acids Res 1993;21(10):2355-61.
7. Roger G.S, Gilchrest BA. The senile epidermis: Enviromental influences on skin ageing and cutaneus carcinogenesis. Brith J Dermatol (supplement) 1990;122 (35):55-60.
8. Nishigori C. UV induced DNA damage in carcinogenesis and its repair. J Dermatol Sci 2000;23 supl.1: s41-S44.
9. Vink AA, Strickland FM, Bucana C, Cox PA, Roza L, Yarosh DB, Kripke ML. Localization of DNA damage and its role in altered antigen-presenting cell function in ultraviolet-irradiated mice. J Exp Med 1996;183(4):1491-500.
10. Kripke ML, Cox PA, Bucana C, Vink AA, Alas L, Yarosh DB. Role of DNA damage in local suppression of contact hypersensitivity in mice by UV radiation. J Exp Dermatol 1996;5(3):173-80.
11. Grewe M, Stege H, Vink A, Klammer M, Ruzicka T, Roza L, Krutmann J. Inhibition of intercellular adhesio nmolecule-1 (ICAM-1) expression in ultraviolet B-irradiated human antigen-presenting cells is restored after repair of cyclobutane pyrimidine dimers. Exp Dermatol 2000;9(6):423-30.
12. Masala G, Ambrogetti D, Assedi M, Giorgi D, Del Turco MR, Palli D. Dietary and lifestyle determinants of mammographic breast density. A longitudinal study in a Mediterranean population. Int J Cancer 2006, 118(7):1782-89.
13. Hashim YZ, Eng M, Gill CI, McGlynn H, Rowland IR. Components of olive oil and chemoprevention of colorectal cancer. Nutr Rev 2005;63(11):374-86.
14. Arief Budiyanto, Ahmed NU, Wu A, Bito T, Nikaido O, Osawa T, Ueda, Ichihashi M. Protective effect of topically applied olive oil against photocarcinogenesis following UVB exposure of mice. Carcinogenesis 2000; 21(11):2085-100.
15. Ichihashi M, Ahmed NU, Arief Budiyanto, Wu A, Bito T, Ueda M, Osawa T. Preventive effect of antioxidant on UV induced skin cancer in mice. J Dermatol Sci 2000; 23 supl: S45-S50.
16. Sapino S, Carlotti ME, Peira E, Gallarate M. Hemp-seed and olive oils: their stability against oxidation and use in O/W emulsions. J Cosme Sci 2005;56(4): 227-51.
17. Lehr HA, van der Loos CM, Teeling P and Gown AM. Complete Chromogen Separation and Analysis in Double Immunohistochemical Stains Using photoshop-based Image Analysis. Histochem Cytochem 1999;47(1):119 25.
18. Bacci S, Nakamura T, Streilein JW. Failed antigen presentation after UVB radiation correlates with modifications of Langerhans cell cytoskeleton. J Invest Dermatol 1996;107(6):838-43.
19. Kölgen W, van Steeg H, van der Horst GT, Hoeijmakers JH, van Vloten WA, de Gruijl FR, Garssen J. Association of transcriptional coupled repair but not global genome repair with ultraviolet B induced langerhan cells depletion and local immunosuppression, J Invest Dermatol 2003:121:751-56.
20. Kölgen W, Both H, van Weelden H, Guikers KL, Bruijnzeel-Koomen CA, Knol EF, van Vloten WA, De Gruijl FR. Epidermal langerhan cells depletion after artificial ultraviolet B irradiation of human skin invivo: Apoptosis versus migration. J Invest Dermatol 2002; 118: 812-17.
21. Hofmann-Wellenhof R, Smolle J, Roschger A, Strunk D, Hubmer M, Hoffmann C, Quehenberger F, Horn M, Kerl H, Wolf P. Sunburn Cell Formation, Dendritic Cell Migration,and Immunomodulatory Factor Production After Solar-Simulated Irradiation of Sunscreen-Treated Human Skin Explants In Vitro. J Invest Dermatol 2004; 123:781-87.
22. Flora Ramona, Arief Budiyanto, Wirohadidjojo YW, The role of SPF in the prevention of UVB induced Langerhan cells depletion, Regional Confference of Dermatology, Bali, 2006.