NOVATEUR PUBLICATIONS
INTERNATIONAL JOURNAL OF INNOVATIONS IN ENGINEERING RESEARCH AND TECHNOLOGY [IJIERT]
ISSN: 2394-3696 Website: ijiert.org
VOLUME 7, ISSUE 6, June-2020

IMPACT OF UV-B RAYS ON PHOTOAGING

PONCOJARI WAHYONO
Faculty of Teacher Training and Education University of Muhammadiyah Malang
ponco1201@gmail.com

ABSTRACT
Photoaging is an aging process that occurs due to various factors from outside the body such as sunlight. Skin changes that occur are not comprehensive. This happens because Photo aging is a process that involves the reduction of collagen and elastin fiber due to UV radiation from the sun, which has a negative effect on characteristics such as wrinkles, pigmentation spots, decreased skin elasticity and rough texture. The aim of the study was to determine the various variants of ultraviolet B dose to damage to collagen in rat skin. 30 rats were divided into 5 groups of 6 rats each. The control group of 4-month-old mice was left to 7 months (D-0), the other groups treated were divided into 4 groups. The four treatment groups were: administration of UV B 90 mJ / cm2 (Group D1) administration of UV B light 110 mJ / cm2 (Group D2), administration of UV-B light 130 mJ / cm2 (group D3) and administration of UV-B light 150 mJ / cm2 (group D4). Treatment was given to each treatment group for 3 months. The experimental design using a completely randomized design with 5 treatments and 6 replications. Data was analyzed by analysis of variance and followed by LSD Test. Collagen type-1 and MMP-1 expression were measured by immunohistochemical techniques and compared with controls. The results showed that significantly different ($P < 0.01$) decreased type-1 collagen in MMP-1 rise. The conclusion of this study is that the increased administration of UVB rays ranging from 130 mJ / cm2 to 150 mJ / cm2 causes the expression of type-1 collagen to decrease and MMP-1 expression increases.

KEYWORDS: Photoaging, UV-B, MMP-1, and collagen type-1

INTRODUCTION
Photo aging is a process that involves the reduction of collagen and skin elastin fibers due to UV radiation from the sun, which has a negative effect on characteristics such as wrinkles, pigmentation spots, decreased skin elasticity and rough texture (Yaar M and Gichrest BA, 2003). Early aging can be inhibited or prevented by avoiding factors that accelerate the process (Fisher GJ. Et al. 1997).
Radiation by the oxidative environment, especially UV, causes damage to the skin. UV exposure for 10-20 minutes causes the hydrogen peroxide level in the skin to be two-fold higher than the original level. Furthermore hydrogen peroxide can quickly trigger the formation of other SORs (Dalle CM and Pathak MA. 1992). Solar UV radiation in living cells can cause various risks of photochemistry such as photooxidation, isomerization photos, and photooxidation. Photooxidation reactions occur due to the release of reactive oxygen species (ROS) in the form of: superoxide anions (O2 ● ·), hydrogen peroxide (H2O2) and hydroxyl radicals (OH ●) by chromophores that absorb ultraviolet light (Kochevar IE, 1995; Jung GE et al. 1995). Skin reactions to UV radiation, including: the formation of free radicals (O2 ● and OH ●), and direct cell death. The patobiological effects of ultraviolet light (UV-A and UV-B) produce free radicals and cause damage to DNA, allegedly free radicals are the main factor that accelerates the premature aging process (Beckman K.B, et al, 1998).
Increased ROS as a result of free radicals because of this UV-B rays can cause an increase in lipid perooksidasi. The high levels of free radicals in the body can be indicated by low enzyme anti-oxidant activity and high malondialdehyde (MDA) (Winarsi, 2007). This ROS compound also plays a role in collagen metabolism, because unless it is able to destroy collagen it can also induce several enzymes that play a role in the degradation of collagen namely matrix metalloproteinase (MMPs), resulting in decreased skin collagen (Dalle CM, and Pathak MA, 1992; Pinnell SR, 2003). The accumulation of reactive oxygen compounds will increase MMP-1 and MMP-3. MMP-1 will break down type-1 collagen, while MMP-3 can break down type IV collagen, proteoglycans, fibronectin and laminin. Damage to collagen types I and IV will result in a decrease in collagen products. This accumulation of collagen reduction is an indicator of skin wrinkles due to the aging process (Pinnel SR, 2003).

Ultraviolet-B radiation plays a major role in changes in procollagen I and III production and increases pro matrix metalloproteinase-1 and -3 enzymes both of which are synthesized and excreted by fibroblast cells (Kaharai & Saaarial-Kere, 1997; Parks & Mecham, 1998, Fisher 2000; Uchida 2000). In vitro studies have shown that UV-B radiation doses of 10-40 mJ / cm2 with 15-30 and 60 seconds of radiation have the potential to reduce the viability of skin type IV-V fibroblasts (Fitzpatrick) (Yulianto I, 2006). The results showed that administration of 15 mJ / cm2 of UV-B radiation in human keratinocyte cultures could increase MMP-1 (Xian-Yong W and Zhi-gong B.I, 2006). The research report shows that 75 mJ / cm2 of UV-B radiation for 72 hours in human dermis culture can increase Kim's MMP-1 expression H.H et al (2005). UV-B radiation 3 times a week for 6 weeks with a size of 130 mj / cm2 in vivo caused an increase in fibroblast damage in mice (Tsukuhara K, et al, 2001 and Vayalil PK, et al, (2004) reported that UV-radiation B with a dose of 90 mJ / cm2 for 2 months with 2 days administration can increase MMP-3 on rat skin in vivo.
The above data is a fact that revealed that UVB rays are harmful to body skin cells, however, it has not been revealed further about the effects of UVB rays on photoaging, especially in decreasing MMP1 and increase in type-1 collagen. As for what we want to see in this study is the effect of various doses of UVB on the expression of MMP-1 and collagen type-1 in the rats' skin from ages 4 to 7 months

METHODS

Research design

This research is a pure experimental study (true experimental) using experimental animals white rats (Rattus norvegicus stran wistar).

Treatment Material

The research material was white rats (Rattus norvegicus stran wistar) which were maintained in the Biochemistry laboratory of the Faculty of Medicine, University of Muhammadiyah Malang. Preparations were made in the Anatomical Pathology Laboratory of Dr. Sutomo Hospital / FK Airlangga University, Surabaya. Painting and immunohistochemical observation of MMP-1, and Type-1 Collagen were carried out by the Lab. Biochemistry-Biomolecular FK Universitas Brawijaya.

Treatment of White Mice

Twenty-five white rats (Rattus norvegicus strain wistar) with 3.5 months of age with an average weight of 220 grams, were acclimatized to the environment of the enclosure in the laboratory for 2 weeks. Rats were randomly divided into 4 treatment groups, with each group as many as 6 rats. D-0 group was controlled (rats without irradiated UVB), group D-1 (rats irradiated with UVB 90 mJ / cm2) group D-2 (rats irradiated with UVB 110 mJ / cm2), group D-3 (mice irradiated with UVB 130 mJ / cm2) and D-4 group (mice irradiated with 150 mJ / cm2 UVB). Mice were switched off together after receiving previous anesthesia with ether on day 90 in the morning for tissue extraction.

Immunohistochemical observations of MMP-1, and type-1 collagen

A glass of paraffin block is immersed in xylol 2 times for 5 minutes each. After that, rehydration was done using serial alcohol (absolute, 96%, 80%, 70%, 5% and 30%) for 5 minutes, respectively. Then rinse in dH2O for 5 minutes.
The slides were washed with PBS pH 7.4 once for 5 minutes. Endogenous peroxide blocking uses 3% H2O2 for 20 minutes. Wash using PBS pH 7.4 three times, for 5 minutes. Specific protein blocking uses 5% FBS containing 0.25% Triton X-100. Wash using PBSpH 7.4 three times, for 5 minutes. Incubation using rabbit anti-polyclonal (MMP-1 and collagen type-1) for 60 minutes. Wash using PBSpH 7.4 three times, for 5 minutes. Incubation using conjugated anti-rabbit HRP for 40 minutes. Wash using PBSpH 7.4 three times, for 5 minutes. Tetesi with DAB (Diamino Benzidine) and incubation for 10 minutes. Wash using PBSpH 7.4 three times, for 5 minutes. Wash using dH20, for 5 minutes. Counterstaining using Mayer Hematoksilin which was incubated for 10 minutes and washed using tap water. Rinse using dH2O and dry air. Mounting uses an entanglement and covers with a glass cover. Observation using a microscope at 400x magnification (Pizem J, Cor A. 2003).

RESEARCH RESULT

Effects of Giving UVB Rays on the Back Skin of Wistar Strain Mice on Type-1 Collagen expression.
The average cell expressing Type-1 Collagen in each treatment group is presented in Figure 1.

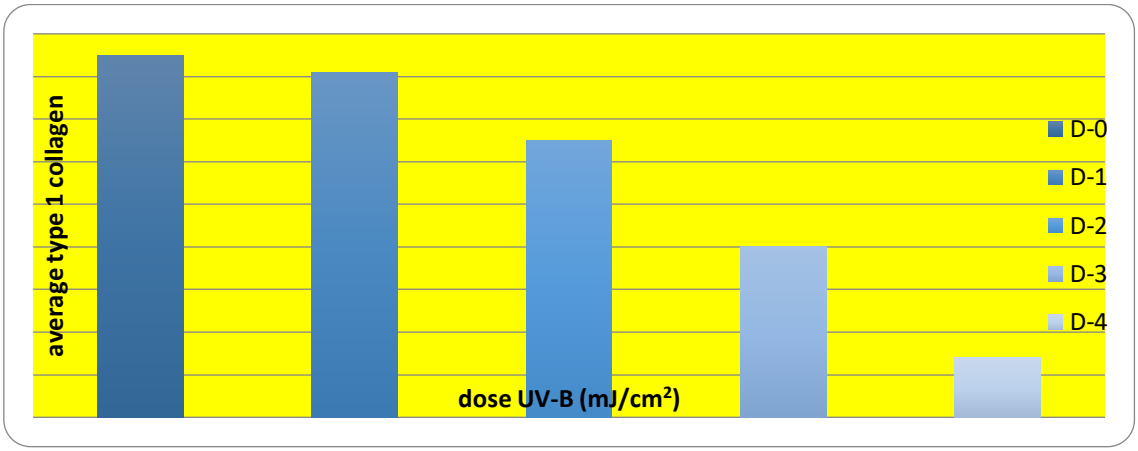

Figure 1 Graph of collagen type-1 expression in skin tissue in various UV B doses

Information

Notation with different letters shows a significant difference in cells that express type-1 collagen which is significant ($p < 0.01$)
D-0: control; D-1: 90 mJ / cm2 of UVB administration; D-2: 110 mJ / cm2 of UVB administration; D-3: administration of 130 mJ / cm2 and D-4 UVB: 150 mJ / cm2 of UVB
Figure 1 shows that the average number of yeng cells expressing the highest type-1 collagen is in the control (D-0), namely: 17.00 ± 1.55, while the lowest average of 150 mJ / cm2 of UVB is: 2, 67 ± 1.21. Oneway Anova statistical test results ($\alpha = 0.01$), showed that there was a significant difference in the number of cells expressing type-1 collagen which was significant ($p = 0,000$) between each treatment group. The LSD further test shows that: D-0 is different from D1. D2, D3 and D4; D-1 is different from D2, D-3 and D-4; D-2 is different from D-3 and D-4; ($p = 0,000$). This shows that the expression of type 1 collagen in skin cells decreases with UVB administration. Linear regression with the equation $y = 18.59-0.09x$ shows a very negative negative correlation ($R = -0,879$), this indicates that the higher the dose of UVB, the less skin cells express type-1 collagen

Effect of Giving UVB Rays on Back Skin of Galist Wistar's Rat for three months on MMP-1 expression

The average cell expressing MMP-1 in each treatment group is presented in Figure 2.

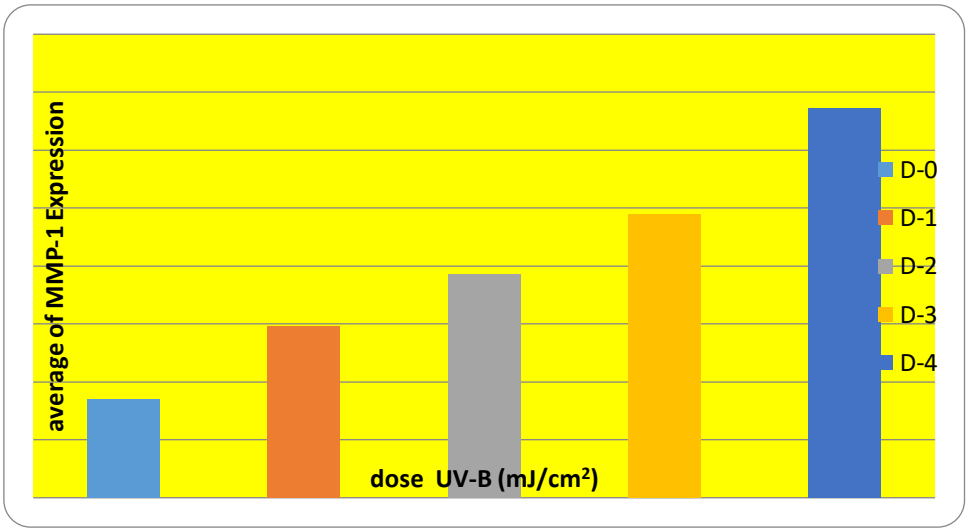

Figure 2 Graph of MMP-1 expression in skin tissue in various doses of UV-B

Information

Notation with different letters shows a significant difference in cells that express significant MMP-1 ($p < 0.01$) D-0: control; D-1: 90 mJ / cm2 of UVB administration; D-2: 110 mJ / cm2 of UVB administration; D-3: administration of 130 mJ / cm2 and D-4 UVB: 150 mJ / cm2 of UVB

Figure 1 shows that the average number of yeng cells expressing the lowest MMP-1 is in the control (D-0), namely: 8.5 ± 1.64, while the highest average for 150 mJ / cm2 UVB is: 33.5 ± 1.97 Oneway Anova Statistical Test Results ($\alpha = 0.01$), showed that there were differences in the average number of cells expressing significant MMP-1 ($p = 0{,}000$) between each treatment group. The LSD further test shows that: D-0 is different from D1. D2, D3 and D4; D-1 is different from D2, D-3 and D-4; D-2 is different from D-3 and D-4; ($p = 0{,}000$). This shows that the expression of MMP-1 in skin cells increases with UVB administration. Linear regression with the equation $y = 5.85 + 0.15x$ shows a very strong positive correlation ($R = 0.887$), this indicates that the higher the dose of UVB, the fewer skin cells that express MMP-1

DISCUSSION

This study wanted to see the effect of tomato extract in preventing collagen damage in the back skin tissue as a result of UVB rays. The process of collagen damage in the aging process is caused by the role of free radicals that are increasing as a result of giving UVB.

MMP-1 degrades collagen at least 70% dry from the weight of the dermis. The aging process increases the activity of MMP-1 in the skin in vivo. Early imbalance between MMP-1 and tissue metalloproteinase 1 / Tissue inhibitors of matrix metalloproteinase (TIMP-1) can increase aging (18). MMP-1 produced by epidermal keratinosites and dermal fibroblasts in response to various stimuli, seems to play an important role in changing the shape of the dermis layer (Twining SS, 1994). Some MMPs are produced during wound healing, such as MMP-3 in the repair of the epidermal layer (Bullard KM, Lund L, Mudgett JS, et. Al, 1999). In living cells, free radicals form on the plasma membrane, also in cell organelles such as peroxisomes, endoplasmic reticulum, mitochondria and cytosols through chain enzymatic reactions that take place through metabolic processes. Free radicals are highly reactive, can cause chemical changes and damage various components such as carbohydrates, nucleotides, lipids, and proteins. In normal circumstances these free radicals can be muted by the body, because naturally the body produces antioxidants, such as catalase and peroxidase dismutase. Increased administration of UVB rays causes radicals to increase while natural antioxidants are insufficient. The result is an imbalance between free radicals produced with existing antioxidants, so that Reactive Oxygen Species (ROS) are formed. ROS plays an important role in collagen metabolism. Reactive oxygen compounds not only directly destroy interstitial collagen but also induce a group of enzymes responsible for the degradation of collagen, namely matrix metalloproteinase (MMPs), resulting in skin collagen loss (Dalle CM, Pathak MA, 1992; Pinnell SR, 2003).

This increase in ROS which then through the MAPK pathway will reduce Extracelluler signal-regulated kinase (ERK) and increase c-Jun Kinase (JNK / p38), which in turn activates the increase in AP-1. Increasing AP-1 will cause an increase in MMP-1 (collagenase). Furthermore, the increase in MMP-1 will activate the decrease in pro-collagen-1. Because the type-1 collagen produced decreases, collagen produced by skin cells will also decrease. This free radical activity and ROS will activate AP-1 through jun kinase via the MAPK pathway (The mitogen-activated protein kinase). Included in this pathway are ERK (Extracelluler signal-regulated kinase), JNK (c-jun N-terminal kinase) and p38. ERK affects jun activity. Activity p38 and ERK can activate AP-1. The three MAPK lines can be activated at the same time. (Outburg S. Joke S., Janneke E., et al., 2005; Ikeda U., Shimpo M., Ohki R., et al, 2000).

CONCLUSION

1. Giving UVB rays has an effect of decreasing the expression of collagen type-1 and an increase in MMP-1 in the skin of mice aged from 4 to 7 months.
2. Giving UVB light at a dose of 130 mJ / cm2 to 150 mJ / cm2 can reduce the expression of collagen type-1 and increase the expression of MMP-1 in the skin of mice aged from 4 to 7 months.
3. In the administration of UVB rays at a dose of 90 mJ / cm2 up to 150 mJ / cm2 aged 4 to 7 months the experimental mice, there is a negative correlation to the expression of collagen type-1 with a regression

equation y = 18,59-0,09x, and there is a positive correlation with the expression of MMP-1 with a regression equation for MMP-1 that is y = 5.85 + 0.15x

REFERENCES

1. Beckman Keneth B. Bruce N. Amies. The Free radical theory of Aging matures. Physiol Rev 2002; 82:47-95.
2. Bullard KM, Lund L, Mudgett JS, et.al. Inpaired wound contraction in stromelysin -1-deficient mice. Ann Surg, 1999; 97: 4052-7.
3. Cenene-Adam K, Campbell JK, Zaripheh S, Jeffery EH, Erdman JW, The tomato as a functional food., J Nutr, 2005; 135:1226-1230
4. Dalle CM, Pathak MA, Skin Photosensitizing agents and the role of reactive oxygen species in photoaging, Photochem photobiol 1992; 14: 105-24.
5. Dalle CM, Pathak MA. Skin photosensitizing agents and the role of reactive oxygen species in photoaging. Photochem Photobiol. 1992;14:105-24.
6. Darmojo RB, Teori Proses Menua, Di dalam: Darmojo RB dan Martono HH, Geriartri (ilmu Kesehatan Usia Lanjut), edisi ke-3. Jakarta: Balai Penerbit Fakultas Kedokteran Universitas Indonesia; 2004; 4-8.
7. Fisher, G. J., and J. J. Voorhees. 1998. Molecular mechanisms of photoaging and its prevention by retinoic acid: ultraviolet irradiation induces MAP kinase signal transduction cascades that induce Ap-1-regulated matrix metalloproteinases that degrade human skin in vivo. J. Invest. Dermatol. Symp. Proc. 3: 61–68.
8. Frank R. De Gruiji. Photocarcinogenesis:UV vs UB Radiation. Skin Pharmacol Appl Skin Physiol 2002; 15: 16-20.
9. Ikeda U., Shimpo M., Ohki R., et al., Fluvastatin Inhibits Matrix Metalloproteinase-1 Expression in Human Vascular Endothelial Cells, Hypertension, 2000;36:325-338)
10. Kochevar Irene E., Molecular and cellular effects of UV radiation relevant to chronic photodamage. In: Gicherst BA. Photodamage, New York: Blackwell Science; 1995.p.52-67
11. Lahmann C, Bergemann J, Harrison G, Young AR. Matrix metalloproteinase-1 and skin ageing in smokers. The Lancet, 2001; 357: 935-6.
12. Outburg S. Joke S., Janneke E., et al. BMC Infectious Diseases, Journal of Translational Medicine. vol. 5:114; 2005; 1471-1486.
13. Pinnell SR,. Cuteneous photodamage, oxidative stres and topical antioxidant protection . J Am Acad Dematol 2003; 48: 1-19.
14. Pinnell SR. Cutaneous photodamage, oxidative stress, and topical antioxidant protection. J Am Acad Dermatol 2003;14:1-19
15. Pizem J, Cor A. 2003, Detection of Apoptosis Cells in Tumour Paraffin Section, Radiol. Onco, 37(4): 225-232
16. Shin MH, Rhie G, Kim YK, Park CH, Cho KH, Kim KHEun HC, Chung JH, H2O2 Accumulation by Catalase Reduction Changes MAP KinaseSignaling in Aged Human Skin In Vivo, Journal Invest Dermatology 125, Seoul, 2 August 2005; 221-229.
17. Twining SS. Regulation of proteolytic activitiy in tissues. Crit Rev Biochem Mol Biol, 1994; 29: 315-83.
18. Wahyono P, Uji Bioaktivitas Antioksidan Ekstrak Buah Tomat (Lycopersicum pyriforme) terhadap Ekspresi Kolagen tipe-1,MMP-1 dan MMP-3 Pada Proses Penuaan Kulit, Laporan Penelitian Fundamental, Lembaga Penelitian Universitas Muhammadiyah Malang, 2008
19. Wahyono P, Aktivitas Antioksidan Ekstrak Buah Tomat (Lycopersicum pyriforme) dalam Mencegah Kerusakan Hepar, Laporan Penelitian Fundamental, Lembaga Penelitian Universitas Muhammadiyah Malang, 2006
20. Wahyono P, Pengaruh Pemberian sari Buah Tomat (Lycopersicum pyriforme) terhadap efek hepatotoksik CCl4 pada tikus Putih, Prosiding Konggres X Ikatan Farmakologi Indonesia, Malang, 15-18 nopember 2000.
21. Winarsi H, Antioksidan Alami dan Radikal Bebas, Penerbit Kanisius; 2007.p. 11-23.
22. Yaar M, Gilchrest BA, Aging of Skin. In: Freedberg IM, Eisen AZ, Wolff K, (eds). Fitzpatrick's dermatology in general medicine 5th ed. New York: McGraw-Hill; 2003.p. 1386-98.