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<table>
<thead>
<tr>
<th>No.</th>
<th>Title</th>
<th>Authors</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>13</td>
<td>The Role of TLR 24 and Characteristics as Signaling of Immune System by Chickens Infected Avian Influenza Virus (Rantam et al.)</td>
<td>Rantam et al.</td>
<td>74-81</td>
</tr>
<tr>
<td>14</td>
<td>Characterization and the Increase of Chicken Interferon-gamma Production as a Measure of T-cell Responses to Eimeria tenella Antigens (Yunus et al.)</td>
<td>Yunus et al.</td>
<td>82-89</td>
</tr>
<tr>
<td>15</td>
<td>Characterization of Specific Protein of T. canis for the Development of Diagnostic by Antibodies Examination of Case Toxocariasis (Kusnito)</td>
<td>Kusnito</td>
<td>90-95</td>
</tr>
<tr>
<td>16</td>
<td>Study of Skin Premature Aging Induced by Ultraviolet B Light Irradiation in Bally/C Mouse Model (Purwandari and Widyarini)</td>
<td>Purwandari and Widyarini</td>
<td>96-103</td>
</tr>
<tr>
<td>17</td>
<td>Pharmacokinetic Profile and Pharmacokinetic/Pharmacodynamic (PK/PD) Parameter of Doxycycline in Broiler Plasma and Tissues after Single Dose Intravenous Administration (Wijayanti et al.)</td>
<td>Wijayanti et al.</td>
<td>104-109</td>
</tr>
<tr>
<td>18</td>
<td>Goat’s Immune Response for Prolactine from Isolation in Moulting Duck (Safiti et al.)</td>
<td>Safiti et al.</td>
<td>110-115</td>
</tr>
<tr>
<td>19</td>
<td>Detection of Enterotoxin Genes of Staphylococcus aureus Isolated from Waste Slaughtered House and Carcass of Chickens (Khussan et al.)</td>
<td>Khussan et al.</td>
<td>116-121</td>
</tr>
<tr>
<td>20</td>
<td>Reaction of Thyroxin, Hematocrit, Haemoglobin on Reducing Feedstuff and Drinking Water (Achmad)</td>
<td>Achmad</td>
<td>122-125</td>
</tr>
<tr>
<td>21</td>
<td>In vitro Anticestode Activity of Painted Nettle Leaves Extract to Hymenolepis microstoma: Observation Using SEM (Ridwan et al.)</td>
<td>Ridwan et al.</td>
<td>126-133</td>
</tr>
<tr>
<td>22</td>
<td>Suckling Behaviour of Bali Calves with Dams Different in Milk Production (Henderiana et al.)</td>
<td>Henderiana et al.</td>
<td>134-139</td>
</tr>
<tr>
<td>23</td>
<td>Comparison between Calipers and Ultrasonography for Measurement of Testicular Volume and Mass in Dogs (Junaidi)</td>
<td>Junaidi</td>
<td>140-146</td>
</tr>
</tbody>
</table>
Study of Skin Premature Aging Induced by Ultraviolet B Light irradiation in Balb/C Mouse Model

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Abstract

The aim of this experiment was to study the process of skin premature aging in mouse model using Ultraviolet B light irradiation. Observed variables were structural changes of skin epidermis, collagen, and elastine by HE, Mallory and Alcian Blue staining. Twenty four weeks old female Balb/c mice were divided into five groups. The first group as the control group was untreated (unirradiated), the rest of the groups were given UVB light irradiation for 5, 10, 15, and 20 weeks. UVB dose was 465 mJ/cm² for 3 minutes. Back hair of mice was shaved before UVB light irradiation. Drinking water was given ad libitum. Treated groups (UVB irradiated mice) showed significant changes particularly in the skin histological structure. Those changes were observed at the fifth to twenty weeks after irradiation. Epidermis layer of skin underwent hyperplasia, hyperkeratosis, inflammation and wrinkled. Tumor nodules identified as squamous cell carcinoma developed at sixteen weeks after UVB irradiation. Collagen fiber was reduced and elastic fiber was accompanied by blue amorphous masses.

Key words: UVB irradiation, premature aging, histological changes of skin.

Introduction

Indonesia has the tropical weather with ample sunlight that highly risks skin from damage or premature aging (Sumaryo, 2000). Skin premature aging is a skin aging process that is faster than normal. Individual aging was highly affected by various factors either external or internal factors. The external factors were mostly genetic factors, diseases such as atherosclerosis, diabetes mellitus, and osteoarthritis while external factors were exposure to sunlight, pollution, cigarette smoke and unheathy diets (Cotran, 1994).

Radiation or exposure to UV may result in changes in epidermis and basal layer that may cause skin to become thin, wrinkled, dry and rough (Makrantonas and Zeoboulis, 2007). Apart from that, UV exposure may cause skin thickening, uneven pigment distribution and the increase of fluid loss through skin, therefore skin looked dry (Fourtanner et al., 1986). Changes in human skin caused by UV exposure were similar with those in mice experimentally exposed to UV (Kligman et al., 1982; Fourtanner et al., 1986).

Skin layer continuously affected by environment particularly UV radiation and high oxygen concentration will get it structure affected and form reactive oxygen species (ROS) (Fuchs, 1998). The formation of ROS in this condition had important role in triggering the emergence of skin diseases such as skin premature aging and skin cancer (Wheeler et al., 1980; Fuchs et al., 1989; Lopez et al., 1998). Photo aging process is highly correlated with sub cats. In this process skin collagen and elastic fiber will be reduced due to sunlight UV exposure therefore skin became metastatic. This occurrence happened on human skin with further chemical changes, including the decrease of water content from 86% at younger ages to 60% at older ages (Spector, 1990). Excessive UV light exposure also may cause skin damage through the emergence of proteolytic enzymes from the formed free radical. These enzymes then broke collagen and connective tissues under skin’s dermis (Lam and Sulistio, 2001).

UV radiation that reaches earth consists of 90-95% UVA and 5-10% UVB. UVA light has a longer wavelength than UVB therefore UVA penetrates deeper into skin and can pass through window glass and also may be able to damage/destroy/ruin/break cellular protein, lipid and saccharides. The damage by UVA can cause necrosis of endothelial cell, dermal blood vessel damage and cancer (Edlich et al., 2004). UVB radiation is called as burning ray. This light...
may cause skin inflammation and erythema and also directly and indirectly induces biological effect, including the formation of photoproduct pyrimidine, trans-cis isomerization uracil: cysteine, ornithine decarboxylase activity induction, DNA synthesis stimulation, free radical formation in skin, photaging and photocarcinogenesis. UVB light may significantly reduced antioxidant power at skin, reduced the ability of skin to protect itself against free radical generated by UV light radiation and also may induce skin cancer (squamous and basal cells carcinoma) because of the damage of DNA (Fisher et al., 1997).

In a previous study, premature aging induced by UV light exposure was caused by the formation of ROS on skin by the exposure to UVB light (290-320 nm wavelength) and UVA (320-400 nm wavelength) (Pinnell, 2003). Albino Skh-1 hairless mice were reported to be used in a study as model animals for premature skin aging that utilized UV exposure (Reeve et al., 2005). In Indonesia this strain of mice is not available therefore in this study Balb/c strain was used as the animal model of premature skin aging in human. Observed variables in this study were the structural changes of collagen and elastin by means of histochimistry technique.

Materials and Methods

Experimental animals
Twenty five female 4-week old Balb/c mice were randomly divided into 5 groups. Except for control group, each group was exposed to UVB for 5, 10, 15, 20 weeks. Mice were obtained from Experimental Animal Development Unit of Integrated Development and Research Department, Gadjah Mada University, fed ERII with ad libitum drinking water. Before UVB exposure back hair was shaved.

UV light radiation
UVB lamp (Dermatology, Medicine Faculty, Gadjah Mada University) (270 - 320 nm wavelength) as the sources of UV light was put on a table at 20 cm from the mice. Room temperature was stabilized using electric fan. UV exposure dose was 465 ml/m² for 3 minutes. UV light exposures were conducted for 5 consecutive days in a week for 5, 10, 15 and 20 weeks.

Skin sample collection
Back skins of mice were necropsied after UVB irradiation for 5, 10, 15 and 20 weeks. Sections of 5x3 cm skin samples was fixed with 10% buffered formalin for cytochemical staining process referenced from standard procedure of Laboratory of Histology and Cell Biology, Faculty of Medicine, Gadjah Mada University and Laboratory of Pathology, Faculty of Veterinary Medicine, Gadjah Mada University.

Hematoxylin and eosin (HE) staining
Back skin tissues fixed in 10% buffered formalin for 24 hours were cut into 3-5 mm thick slices. These slices were dip into increasing concentrations of ethanol i.e. once in 80% ethanol, twice in 95% ethanol and three times in absolute ethanol for 60 minutes each. Tissues were then embedded in paraffin and cut into 4-5μm thick slices using microtome. Slices were mounted on object glass smeared with Mayer’s egg albumin and allowed to dry. Hematoxylin and Eosin staining was performed after deparaffinization in xylol (Anonymous, 1957).

Mallory staining
The 5 μm tissue slices were deparaffinized by immersion into xylene, absolute alcohol, 95% alcohol and flowing water. Tissues were soaked in iodine alcohol solution for 5-10 minutes and rinsed with water. Proper iodine removal was performed using 5% sodium thiosulfate solution for 5 minutes and then rinsed with flowing water for 10-20 minutes. Staining was conducted for 12 to 24 hours in phosphotungstic acid hematoxylin solution. Differentiation was performed using 95% alcohol and then slides were observed under microscope. After that slides were rinsed twice in absolute alcohol and twice in xylene and then covered with Entellan.

Alcian blue staining
Xylene deparaffinized 5 μm skin samples were hydrated with aqua bidest, then immersed in 3% acetic acid for 3 minutes and alcian blue solution for 30 seconds. Slides were rinsed with flowing water for 2 minutes and soaked in nuclear-fast red for 5 minutes then rinsed with water. Dehydration was performed using increasing concentration of alcohol. Slides were cover slipped and observed under binocular for mucopolysaccharide acid that stained blue with pink nucleus.

Analysis design
Skin histopathological changes visualized by HE, Mallory and Alcian Blue staining were analyzed as qualitative descriptive study on epidermis thickness. Anova was followed by T test (significans level 99 %). Duncan Multiple Test and semi-qualitative on collagen and elastin.

Results and Discussion
The observation of macroscopic changes on UVB irradiated and unirradiated control groups mice skins were performed daily. Back skin sample
collection were done by necropsies at 5, 10, 15 and 20 weeks after irradiation according to their treatment group. Based on macroscopic observations of skin UVB irradiated for 5 weeks, 10 weeks, 15 weeks and 20 weeks compared to those of the nonirradiated control group alterations were found in the skin of mice from each group. The skin of control mice appeared thin, elastic and smooth meanwhile in mice irradiated (dosage 465 mJ/cm² for 3 minutes) for 5, 10, 15 and 20 weeks showed alterations in their skin which varied from redness, thickening, wrinkles, toughness to the formation of tumor nodules in 20 weeks irradiated group. Tumor nodules started appearing at week-16 after exposure. Macroscopic changes at the back skin of the mice can be seen in Fig. 1. Back skin of control mouse looked smooth, thin and elastic (Fig. 1A). Back skin of mice irradiated for 5 weeks showed redness, thickening and wrinkles as can be seen in Fig. 1B. Macroscopic alterations at the back skin of mice irradiated for 10 weeks were almost similar to those irradiated for 5 weeks where the skin looked reddish, thickened and wrinkled. Back skin of mice UV irradiated for 15 weeks appeared thick, tough and rough as can be seen in Fig. 1C. Skin tumor nodule was formed in the group that was irradiated for 20 weeks (Fig. 1D).

Tumor nodules (diameter ranged between 0.8 and 1.1 cm) started appearing 16 weeks after UV exposure in three mice. Tumor nodules were identified as squamous cell type carcinoma which was characterized with nodules, hyperkeratosis and necrosis at the central part of the nodule. Acute UVB irradiation may cause sunburn and inflammation reaction while longer irradiation may cause alteration of skin such as dan wrinkles, turgidity and elasticity decreases, pigmentation and the formation of new vessels (Matsumura and Ananthaswamy, 2002). In some research model chronic irradiation caused skin premature aging and squamous cell carcinoma (Cooper et al., 1993).

Microscopically skin alterations were distinctly seen by preparation staining using Hematoxylin and Eosin and from the measurement of epidermis thickness under micrometer microscope. Skin that was UV irradiated showed thickening of epidermis, acanthosis, infiltrative growth of epidermis cells towards the dermis, and keratin pearl accompanied with inflammation reaction. Meanwhile, unirradiated skin appeared thin. This macroscopically feature of the alteration can be seen in Fig. 2. Epidermis thickness measurement values are presented in Table 1.

The measurements of epidermis thickness showed differences among each treated and control groups. ANOVA showed significant differences (p<0.01) on epidermis thickness between treated and control group (unirradiated). Epidermis thickening started at week-5 and still increasing at week-20. In some area, thickness was not measurable because of infiltrative growth of skin tumor (Squamous cell carcinoma). Duncan’s Multiple Range Test on the increase of epidermis thickness in each treatment group (5, 10, 15 and 20 weeks) showed significant difference at p<0.01 and an interaction between age and UV irradiation.

Microscopically features of normal skin epidermis layer are thin complex squamous epithelial cells and loose connective tissue, meanwhile irradiated skin underwent thickening of epidermis layer, hyperkeratosis and inflammation reaction from week-5 to week-20. At week-15 epidermis cells had undergone proliferation towards dermis or what is so called acanthosis and advanced towards the development of squamous cell carcinoma (skin cancer), at week-16. These microscopically changes were in line with the macroscopically visual changes where the skin appeared thickening, reddish with tough consistency.

Skin alteration due to a chronic UVB irradiation may cause skin oxidative damage, immunosuppressant and skin cancer. This was caused by qualitative and quantitative UV light photon absorption by skin layers accounted for the growth of connective tissue therefore ROS was clinically manifested as wrinkled, thick and fragile skin, and the impairment of wound healing at high degree skin damage (Ma et al., 2001).

<table>
<thead>
<tr>
<th>UVB Irradiation</th>
<th>Epidermis Thickness (Mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>21 ± 0.2</td>
</tr>
<tr>
<td>5 weeks</td>
<td>302.8 ± 193.8</td>
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<tr>
<td>10 weeks</td>
<td>305.0 ± 112.1</td>
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<tr>
<td>15 weeks</td>
<td>465.0 ± 32.8</td>
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<td>20 weeks</td>
<td>660.0 ± 100.3</td>
</tr>
</tbody>
</table>

Table 1. Epidermis Thickness (Mean ± SD) of Skin that was Chronically UVB Irradiated for 5, 10, 15, 20 Weeks
At week-16 there was a growth towards the formation of tumor nodules on skin. At week-20 when necropsy was performed, nodules diameter were at a range of 0.8-1.1 cm. Nodules were identified as squamous cell carcinoma. Identification was indicated by epidermis cell proliferation towards the dermis with infiltrative growth and the formation of carcinoma pearl (trapped keratin) among tumor cells. According to Ichihashi et al., (2003) irradiation of UVB as the light component that reaches earth surface may cause DNA damage especially at cyclobutane pyrimidine dimmers (CPDs) and (6-4) photoproduce which induced the mutation of epidermis to develop into cancerous cells in skin. Factors caused skin cancer by UV irradiation was DNA damage and immunosuppressant (Alberts and Hess, 2005).

Squamous cell carcinoma is a malignant tumor that may develop on skin or mucous membrane with squamous epithelial cells. This type of cancer involved greatly varied cell size such as adenoid squamous cell carcinoma and spindle cell tumor type which were aggressive and sometimes were metastasis. The development of squamous cell carcinoma was correlated with the frequency of irradiation, while basal cell carcinoma (BCC) and cutaneous malignant melanoma (CMM) were correlated with a brief irradiation (Kanoko et al., 2000).

Microscopically examination using Mallory staining was aimed to observe collagen growth in dermis. Dermis layer of unirradiated skin showed distinct and organized growth of collagen (Fig. 3A) meanwhile UV irradiated skin showed a reduction of collagen (Figs. 3B, C, D). Kligman and Lavher. (1988) stated that UV irradiation cause thickening of dermis, reduction of collagen and unorganisation of collagen. Collagen was replaced with elastic microfilament and damaged collagen was turned into amorphous masses.

UVB irradiation may cause the inhibition of collagen synthesis and the increasing collagen damage in dermis layer of skin and reduce transforming growth factor β (TGF-β) through transforming growth factor-β type II receptor (TGF-βRII) which is a potential stimulant in the expression and accumulation of type I procollagen (Quan et al., 2004). Besides, UV irradiation causes loss of hyaluronic acid and reduction of HA synthase (HAS1), (HAS2) and (HAS3) which express MENA as well as the reduction of fibroblast proliferation on dermal papilla (Stephan et al., 2007). Chronic UVB irradiation causes damage of connective tissue and extra cellular matrix (ECM) in skin that resulted in the reduction of collagen synthesis and photo aging (Fisher et al., 1997).

Microscopically examination using Alcian Blue staining was aimed to observe elastin growth in dermis. Unirradiated skin showed distinct growth of elastin in dermis layer (Fig. 4A), meanwhile irradiated skin showed a reduced elastin growth. In the group that was irradiated for 20 weeks elastin was totally disappeared and replaced by tumor cells.

Chronic and continuous UV irradiation causes the damage of connective tissue and ECM. In collagen and elastic fiber damage ECM was reformed through metalloproteinase matrix activation (Fisher et al., 1997). In photo aging proteoglycans (PGs) and glycosaminoglycan (GAGs) level fast reduced and causes the damage of skin collagen fiber (Kligman and Lavher, 1988).

GAG is a mucopolysaccharide compound that was produced because of collagen degeneration process of dermis to form granular amorphous materials. After Mowry’s staining, material from mice that were not exposed to UV was seen as substance located among collagen cells which were stained weak blue (Reeve et al., 2005). GAG expression will increase at the middle part up to the lower part of dermis, not only intercellular but also intracellular at collagen tissue of the mice that were exposures chronically with UV light (Reeve et al., 2005). The above histopathology changes has been reported in previous studies (Kligman et al., 1982; Fourtaine et al., 1986).

Changes at mice back skin resulted from exposure to UV occurred because of the loss of hyaluronic acid in papillary dermis and the down regulation of hyaluronic acid (HA) synthase (HAS1), HAS2 and HAS3, transforming growth factor β (TGF-β) and also the TGF-β receptor which were highly influential in skin turgidity and elasticity (Tanimi et al., 2005). In epidermis HA played important roles in regeneration and wound healing (Sator et al., 2004) as well as photo aging (Brulé et al., 1984).

Conclusions

This study showed that UV irradiated skin microscopically underwent histological structure alteration that appeared since week-5 after exposure in the form of epidermis hyperplasia, hyperkeratosis and wrinkles. Tumor nodules which histological were squamous cell carcinoma appeared at week-16 after exposure. Microscopically alteration observed was the reduction of collagen and elastic fibers accompanied with bluish amorphous mass. Eubl/c mice can be utilized as model animals of premature aging of skin from UV irradiation.
Figure 1. Macroscopic changes of mice back skin: A. Mouse from control group showed smooth, thin back skin (arrow); B. Irradiation for 5 weeks resulted in redness, thickening and wrinkles on skin (arrow); C. Irradiation for 15 weeks resulted in thickening, hardening and rough skin (arrow); D. Irradiation for 20 weeks resulted in thickened, hard, rough skin, and nodule formation (arrow).

Figure 2. Microscopically alteration of epidermis layer of skin: A. Epidermis layer appear thin (arrow, H&E, 100x); B. Group that was UV irradiated for 5 weeks showed thickening of epidermis and hyperkeratosis (arrow); C. UV irradiation for 15 weeks resulted in the proliferation of epidermis layer towards the demnis and hyperkeratosis (arrow); D. UV irradiation for 20 weeks resulted in proliferation of epidermis towards the dermis layer, actively dividing nuclei and the formation of keratin pearl (arrow, H&E, 400x).
Figure 3. Microscopically changes of skin of control mice; A. Distinct and well organized collagen fiber (arrow, Mallory, 100x). B. Collagen fiber underwent reduction and unorganized in the group that was UV irradiated for 10 weeks; C. Collagen fiber appeared thin and unorganized in the group that was UV irradiated for 15 weeks; D. Tumor nodules growth with keratin pearl and unorganized collagen in the group that was UV irradiated for 20 weeks (arrow, Mallory, 400x).

Figure 4. Macroscopically changes of skin elastin; A. Control group shows distinct elastin in dermis (arrow, Alcian Blue, 100x); B. Elastin tissue was reduced in the dermis of week-10 group (arrow); C. Elastin tissue was reduced in the dermis of 15-week exposure group (arrow); D. Elastin tissue was disappeared, replaced by tumor cells in the dermis of 20-week exposure group (arrow, Alcian Blue, 400x).
References


