

RINGKASAN

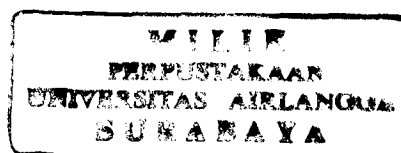
Pengaruh Kadar Melanin Terhadap Terjadinya Akumulasi Kolagen Pada Keloid

David S Perdanakusuma

Keloid adalah suatu parut abnormal yang terjadi akibat proliferasi abnormal dari fibroblas di dalam lapisan dermis kulit, merupakan respon penyembuhan luka yang berlebihan dan berhubungan dengan deposisi kolagen yang berlebih pada jaringan parut. Keloid banyak ditemukan pada orang kulit berwarna dengan pigmen melanin banyak. Semakin gelap seseorang akan semakin besar peluang terjadinya keloid. Pada penderita albino, yaitu suatu keadaan tidak ada atau minimalnya pigmen melanin dinyatakan tidak pernah menderita keloid. Melanin merupakan biopolimer kompleks dalam jaringan kulit normal yang di biosintesis di melanosom dalam melanosit. Melanin merupakan pigmen yang paling menentukan warna kulit berbagai ras di dunia. Melanin bersifat asam, ditemukan dalam melanin adanya fosfatase asam dan melanin banyak mengandung asam karboksilat.

Tujuan penelitian ini adalah mengungkap mekanisme terjadinya akumulasi kolagen pada peningkatan kadar melanin. Dilakukan penelitian eksperimental laboratorik dengan pendekatan biokimia menggunakan sampel penelitian ($n=73$) terdiri dari melanin *Sepia officinalis* (Sigma[®] M 2649), kolagen tipe 1 *insoluble bovine achilles tendon* (Sigma[®] C 9879) dan enzim kolagenase (Sigma[®] EC 3.4.24.3). Eksperimen pertama mengukur pH melanin berbagai kadar (1, 10, 30, 70 dan 90 mg yang dilarutkan dalam 1 ml H₂O₂ 1%) dengan menggunakan pH meter. Eksperimen kedua melakukan reaksi kolagen dengan enzim kolagenase pada berbagai pH (optimal 7,5; 7,2; 7,1; 6,9 dan 6,8), hasil absorbansi dibaca dengan *Elisa reader*. Eksperimen ketiga melakukan reaksi kolagen dengan enzim kolagenase yang ditambahkan melanin berbagai kadar (1, 10, 30, 70 dan 90 mg), hasil absorbansi dibaca dengan *Elisa reader*. Tahap berikutnya melakukan penelitian observasional *cross-sectional* pada penderita keloid dengan sampel penelitian ($n = 30$; 10 pria, 20 wanita, berusia antara 10-45 tahun) diambil dari populasi penderita keloid yang datang ke poliklinik Bedah Plastik RSUD Dr. Soetomo. Terhadap jaringan keloid dilakukan pengukuran pH jaringan keloid segera setelah operasi dengan menggunakan pH meter, setelah itu jaringan dibuat preparat histopatologi dan dilihat dengan menggunakan mikroskop kandungan melanin dan kolagennya.

Data penelitian dianalisis menggunakan *t-test* dan analisis regresi. Hasil eksperimen menunjukkan ada perbedaan yang signifikan antara hasil absorbansi pH $\leq 7,2$ dengan pH $>7,2$ ($p < 0,05$) dan tampak dari hasil eksperimen tidak ada perbedaan antara melanin dengan pH ($p > 0,05$). Hasil pengukuran pH melanin berbagai kadar didapatkan semakin meningkat kadar melanin akan semakin menurun pHnya ($p < 0,05$). Hasil absorbansi reaksi kolagen dengan enzim kolagenase pada berbagai pH didapatkan semakin lebih rendah dari pH 7,5 akan semakin menurun aktifitas degradasi ditunjukkan dengan nilai absorbansi (sisa kolagen) yang semakin



meningkat ($p < 0,05$). Hasil absorbansi reaksi kolagen dengan enzim kolagenase yang ditambahkan melanin berbagai kadar tampak tidak ada pengaruh yang signifikan ($p > 0,05$), hal ini menunjukkan bahwa melanin tidak mengganggu aktifitas degradasi kolagen secara langsung tetapi secara tidak langsung melalui mekanisme penurunan pH.

Pada penelitian observasional didapatkan seluruh jaringan keloid mempunyai $pH \leq 7,2$. Didapatkan semakin tinggi kandungan melanin di jaringan keloid akan semakin rendah pHnya ($p < 0,05$). Semakin rendah pH akan semakin tinggi kepadatan kolagennya ($p < 0,05$). Kandungan melanin yang semakin meningkat serta pH yang semakin menurun akan meningkatkan kepadatan kolagen ($p < 0,05$).

Hasil penelitian eksperimental dan observasional didapatkan bahwa melanin berpengaruh terhadap terjadinya akumulasi kolagen melalui mekanisme penurunan pH menjadi asam yang akan mengganggu enzim kolagenase untuk mendegradasi kolagen.

SUMMARY

The Effect of Melanin Concentration on Collagen Accumulation in Keloid

David S Perdanakusuma

Keloid is an abnormal scar occurring as a result of an aberrant proliferation of fibroblasts in the skin dermal layer, as an exorbitant wound healing response and is associated with excessive collagen deposition in scar tissue. Keloid is often found among colored-skin people possessing abundant melanin pigment. The darker the skin of a person, the greater the chance one has to suffer from keloid. An albino is defined to be a condition where absent or minimal melanin pigment is found and it is stated that a human albino never experiences keloid formation. Melanin is a biopolymer complex in normal skin tissue which is biosynthesized in melanosomes within melanocytes. Melanin is the most crucial pigment in determining skin color of the various races in the world. Melanin is acidic, containing acid phosphatase and ample carboxylic acid.

The objective of this research is to uncover the mechanism whereby collagen accumulates in response to increased melanin concentration. An experimental laboratory research was performed applying biochemistry approach, on a total research sample (n=73) which consisted of *Sepia officinalis* melanin (Sigma® M 2649), insoluble bovine achilles tendon collagen type 1 (Sigma® C 9879) and collagenase enzyme (Sigma® EC 3.4.24.3). The first experiment measured pH of melanin in various concentrations (1, 10, 30, 70 and 90 mg dissolved in 1 ml H₂O₂ 1%) employing a pH meter. A second experiment carried out the reaction between collagen and collagenase enzyme under various pH (optimal 7,5; 7,2; 7,1; 6,9 and 6,8) and the absorbance values were evaluated using an Elisa reader. A third experiment carried out a reaction between collagen and collagenase enzyme to which was added melanin in various concentrations (1, 10, 30, 70 and 90 mg) where the absorbance values were evaluated with an Elisa reader. In the subsequent stage, a cross-sectional observational research was performed on the following research sample (n=30; 10 males, 20 females, age ranged between 10 - 45 years old), which was obtained from a population of keloid patients visiting the Plastic Surgery outpatient clinic in Dr. Soetomo Hospital. Immediately after surgery, pH measurement of keloid tissue was performed employing a pH meter. Next, a histopathologic specimen was developed from the tissue and melanin and collagen concentrations were evaluated under the microscope.

Research data were analyzed using the t-test and regression statistical methods. The results of this experiment demonstrated that there was significant difference between results of absorbance under pH $\leq 7,2$ and pH $> 7,2$ (p < 0,05) and there was no difference between melanin and pH (p > 0,05). The results of pH measurements of melanin in various concentrations revealed that the greater the melanin concentration the less pH it had (p < 0,05). The results of absorbance in the

ABSTRACT

The Effect of Melanin Concentration on Collagen Accumulation in Keloid

David S Perdanakusuma

This research arises from the revelation which demonstrates that colored skin people have a high incidence of keloid, in contrast to albino people who never experience this entity. Keloid is an abnormal scar as the end product of wound healing process, characterized by collagen accumulation. Melanin is the most important pigment which determines variations in the skin color of the various races in the world. The mechanism of the increase in melanin concentration in relation to collagen accumulation will be elucidated, in its association with the underlying process in keloid formation among colored skin people. After exploring a wide range of literature and predicting the possible relationship between melanin and keloid it is assumed that pH is the factor bridging the relationship between melanin and keloid.

An experimental laboratory research was performed, employing the principle of biochemistry reaction between collagen substrate and collagenase enzyme. Experiment was carried out to observe various concentration of melanin pH, collagen-collagenase reaction under various pH conditions, collagen-collagenase reaction in response to addition of different concentrations of melanin (n=73). A cross sectional observational research was also performed on keloid tissue, evaluating tissue pH, melanin concentration and collagen concentration (n = 30).

Research data were analyzed using t-test and regression statistical method. The results of this experiment demonstrate that there is a significant difference between the results of absorbance under $\text{pH} \leq 7,2$ and $\text{pH} > 7,2$ ($p < 0,05$) and there is no difference between the reactions of collagen-collagenase under various pH and the reaction collagen-collagenase in various melanin concentration ($p > 0,05$). Melanin concentration increases in proportion to a decrease in its pH ($R^2 = 0,660$; $p = 0,000$). A decrease in pH leads to a decrease in collagen degradation ($R^2 = 0,190$; $p = 0,033$). In a reaction where $\text{pH} \leq 7,2$ then $R^2 = 0,255$; $p = 0,046$. Melanin concentration increase gives no effect on collagen degradation ($R^2 = 0,000$; $p = 0,996$), because an assay buffer is used in this reaction which makes pH relatively stable at 7,5. The observational research demonstrates that all keloid tissues indicate a pH of $\leq 7,2$. Melanin increases in proportion to a decrease in its pH ($R^2 = 0,673$; $p = 0,000$). pH decrease causes an increase in collagen concentration ($R^2 = 0,713$; $p = 0,000$). Melanin concentration increase will result in increased collagen concentration ($R^2 = 0,866$; $p = 0,000$). An increase in melanin concentration and a decrease in pH will progressively increase collagen concentration ($R^2 = 0,866$; $p = 0,000$).

This research concludes that melanin plays a role in collagen accumulation by decreasing the pH that eventually disrupts collagen degradation process.

Key words : keloid, melanin, pH, collagen synthesis, collagen degradation, collagenase.