ABSTRAK

Pendahuluan. Penyakit kardiovaskular (KV) lebih sering menyerang subyek laki-laki daripada perempuan. Hal ini menimbulkan dugaan adanya peran hormon androgen, khususnya testosteron, dalam kejadian KV. Kejadian KV seringkali diawali dengan adhesi, aktivasi, dan agregasi trombosit. Endotel berperan mencegah aktivasi dan agregasi trombosit, antara lain melalui sintesis dan pelepasan prostasiklin (PGI_2). PGI_2 akan meningkatkan cyclic adenosine monophosphate (cAMP) trombosit dan menurunkan kalsium intratrombosit. Sintesis PGI_2 memerlukan enzim siklooksigenase (COX), khususnya COX-2. Endotel juga berperan dalam adhesi trombosit pada dinding pembuluh darah. Ekspresi *intercellular adhesion molecule*-1 (ICAM-1) oleh endotel diperlukan untuk *firm adhesion* trombosit. Interaksi reseptor *cluster of differentiation*-40 (CD40) endotel dengan CD40 *ligand* (CD40L) pada trombosit yang teraktivasi meningkatkan ekspresi ICAM-1. Hiperglikemia diketahui meningkatkan reaktivitas trombosit, mengaktivasi sel endotel, menurunkan sintesis prostasiklin, dan meningkatkan ekspresi ICAM-1 endotel. Testosteron diperkirakan mempengaruhi fungsi endotel dengan adanya reseptor androgen dan enzim-enzim metabolisme testosteron pada sel endotel, antara lain 5α-reduktase yang mengkatalisis perubahan testosteron menjadi dihidrotestosteron, dan aromatase yang mengkatalisis perubahan testosteron menjadi estradiol. Estradiol akan berikatan dengan reseptor estrogen (ER) pada sel endotel. Muncul pertanyaan apakah testosteron menghambat agregasi trombosit melalui ekspresi COX-2 dan ER-β endotel dan potensi adhesi trombosit pada sel endotel melalui ekspresi ICAM-1 dan CD40 endotel dalam lingkungan glukosa normal atau glukosa tinggi.

Metode. Telah dilakukan penelitian eksperimental laboratoris *in vitro*, percobaan faktorial 2x4 dengan rancang bangun *randomized block* dan perbandingan hasil pengukuran purna uji (*post test only*) dengan kelompok pembanding. Subyek penelitian adalah kultur sel endotel dari vena umbilikalis manusia (*human umbilical vein endothelial cells*) dan suspensi trombosit pada plasma kaya trombosit (*platelet rich plasma*) dari subyek laki-laki dewasa muda sehat. Variabel bebas adalah testosteron dan lingkungan glukosa. Testosteron diberikan dalam 4 *level*, yaitu 0 nM, 1 nM, 10 nM, dan 100 nM, sedangkan lingkungan glukosa diberikan dalam 2 *level*, yaitu glukosa normal 5,6 mM dan glukosa tinggi 22,4 mM. Variabel terikat adalah persentase agregasi trombosit, konsentrasi cAMP trombosit, ekspresi COX-2, ER-β, ICAM-1, dan CD40 endotel. Agregasi trombosit diukur dengan metode turbidimetri, konsentrasi cAMP trombosit dan ekspresi ICAM-1 dan CD40 endotel diukur dengan metode *enzyme-linked immunosorbent assay* (ELISA), dan ekspresi COX-2 dan ER-β endotel diukur dengan metode imunositokimia,. Data dianalisis dengan uji t tidak berpasangan, ANOVA satu jalan, dan analisis varian untuk desain faktorial 2x4.

Hasil. Paparan testosteron pada sel endotel dalam lingkungan glukosa normal atau glukosa tinggi tidak mempengaruhi agregasi trombosit secara bermakna. Konsentrasi cAMP trombosit yang diinkubasi sel endotel yang terpapar

testosteron dalam lingkungan glukosa normal lebih tinggi secara bermakna daripada trombosit yang diinkubasi sel endotel dalam lingkungan glukosa normal tanpa testosteron. Konsentrasi cAMP trombosit yang diinkubasi sel endotel yang terpapar testosteron dalam lingkungan glukosa tinggi lebih rendah daripada trombosit yang diinkubasi sel endotel yang terpapar lingkungan glukosa tinggi tanpa testosteron. Ekspresi COX-2 endotel yang terpapar testosteron dalam lingkungan glukosa normal lebih tinggi secara bermakna daripada sel endotel yang terpapar lingkungan glukosa normal tanpa testosteron. Ekspresi COX-2 endotel yang terpapar testosteron dalam lingkungan glukosa tinggi lebih rendah secara bermakna daripada sel endotel yang terpapar lingkungan glukosa tinggi tanpa testosteron. Ekspresi ER-β endotel yang terpapar testosteron dalam lingkungan glukosa normal lebih tinggi secara bermakna daripada sel endotel yang terpapar lingkungan glukosa normal tanpa testosteron. Ekspresi ER-β endotel yang terpapar testosteron dalam lingkungan glukosa tinggi tidak berbeda bermakna dengan sel endotel yang terpapar lingkungan glukosa tinggi tanpa testosteron. Ekspresi ICAM-1 endotel yang terpapar testosteron dosis bertingkat dalam lingkungan glukosa normal lebih rendah daripada sel endotel yang terpapar lingkungan glukosa normal tanpa testosteron. Ekspresi ICAM-1 endotel yang terpapar testosteron dosis 10 nM dalam lingkungan glukosa tinggi lebih tinggi daripada sel endotel yang terpapar dalam lingkungan glukosa tinggi tanpa testosteron, atau dengan testosteron 1 nM, atau dengan testosteron 100 nM. Paparan testosteron pada sel endotel dalam lingkungan glukosa normal atau glukosa tinggi tidak mempengaruhi ekspresi CD40 secara bermakna.

Kesimpulan. Dalam lingkungan glukosa normal, testosteron berpotensi menghambat agregasi trombosit melalui peningkatan ekspresi COX-2 dan ERβ endotel. Dalam lingkungan glukosa tinggi, testosteron cenderung meningkatkan agregasi trombosit melalui penurunan ekspresi COX-2 endotel. Dalam lingkungan glukosa normal, paparan testosteron berpotensi menghambat adhesi trombosit pada sel endotel melalui penurunan ekspresi ICAM-1 endotel. Dalam lingkungan glukosa tinggi, paparan testosteron dosis fisiologis pada sel endotel meningkatkan potensi adhesi trombosit pada sel endotel melalui peningkatan ekspresi ICAM-1 endotel.

Kata kunci. Testosteron, hiperglikemia, endotel, agregasi trombosit, adhesi trombosit, studi *in vitro*.

ABSTRACT

Introduction. Cardiovascular (CV) diseases are more frequently occurred in age-adjusted male subjects than female ones. It is supposed that androgen hormone-especially testosterone (T)-contributes to the CV events. CV event is initiated by platelet adhesion, activation, and aggregation. Endothelial cell (EC) prevents platelet activation and adhesion by synthesizing and releasing thromboregulator agents, such as nitric oxide and prostacyline (PGI_2). PGI_2 increases platelets cyclic adenosine monophosphate (cAMP) and decreases intraplatelet calcium concentration. In synthesizing PGI_2, EC needs cyclooxygenase (COX) enzyme, especially COX-2 isomer. EC also plays a role in platelet adhesion through intercellular adhesion molecule-1 (ICAM-1) expression, which is increased by interaction between CD40 of EC and CD ligand (CD40L) of activated platelets. Hyperglycemia can increase platelet reactivity, activate EC, decrease PGI_2 and induce expression of ICAM-1. It is suggested that T modulates the function of EC since it contain androgen receptor and enzymes that metabolize T, such as 5α-reductase, which catalyzes conversion of T into dihydrotestosterone, and aromatase, which catalyzes conversion of T into estradiol. Estradiol binds to estrogen receptor (ER) in EC. Thus, it is interesting to know whether T inhibits platelets aggregation mediated by COX-2 and ER-β in endothelial cells and platelets adhesion to endothelial cells mediated by ICAM-1 and CD40 in endothelial cells in either normoglucose or high glucose environment.

Methods. This was an in vitro laboratory study using 2x4 factorial-randomized block-posttest only control group design. Study subject was culture of EC derived from human umbilical vein endothelial cells (HUVEC) and platelet suspension (platelet rich plasma; PRP) from healthy male subjects. Independent variables were T that was given in 4 doses: 0 nM, 1 nM, 10 nM, and 100 nM, and glucose environment that was given in 2 levels: normoglucose (NG) 5.6 mM and high glucose (HG) 22.4 mM. Dependent variables were percentage of platelet aggregation, concentration of platelet cAMP, expression of endothelial COX-2, ER-β, CD40, and ICAM-1. Platelet aggregation was measured using turbidimetri method, concentration of platelet cAMP and expression of endothelial CD40 and ICAM-1 were measured using enzyme-linked immunosorbent assay (ELISA), whereas expression of endothelial ER-β and COX-2 were measured using immunocytochemistry. Data were analyzed using independent t-test, one-way ANOVA, and analysis of varians for 2x4 factorial design. *P* value <0.05 was considered significantly different.

Results. EC exposed to T-and-NG or T-and-HG did not influence significantly platelet aggregation as compared EC exposed to NG-only or HG-only did, respectively. Concentration of cAMP in PRP after incubated with EC exposed to T-and-NG was higher than in PRP after incubated with EC exposed to NG-only. Concentration of cAMP in PRP after incubated with EC exposed to T-and-HG was lower than in PRP after incubated with EC exposed to HG-only. COX-2 expression in EC exposed to T-and-NG was significantly higher than in EC

exposed to NG-only. COX-2 expression in EC exposed to T-and-HG was significantly higher than in EC exposed to HG-only. ER-β expression in EC exposed to T-and-NG was significantly higher than in EC exposed to NG-only. ER-β expression in EC exposed to T-and-HG did not significantly differ with in EC exposed to HG-only. ICAM-1 expression in EC exposed to T-and-NG was lower than in EC exposed to NG-only. ICAM-1 expression in EC exposed to T 10 nM-and-HG was lower than in EC exposed to HG-only or to T 1 nM-and-HG or to T 100 nM-and-HG. CD40 expression in EC exposed to T-and-NG or T-and-HG did not significantly differ with in EC exposed to NG-only or HG-only, respectively.

Conclusion. In normoglucose environment, testosterone is potential to inhibit platelet aggregation by inducting endothelial COX-2 and ER-β expression. In high glucose environment, testosterone is potential to increase platelet aggregation by depressing endothelial COX-2 expression. In normoglucose environment, testosterone is potential to inhibit platelet adhesion to endothelial cells by suppressing endothelial ICAM-1 expression. In high glucose environment, testosterone in physiological dose is potential to facilitate platelet adhesion to endothelial cells by enhancing ICAM-1 expression.

Keywords. Testosterone, hyperglycemia, endothelial cells, platelet aggregation, platelet adhesion, an in vitro study.

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Demikianlah daftar riwayat hidup ini saya buat dengan sebenarnya.

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