The 1th International Conference of Medical and Health Sciences 2015

HIGH GLUCOSE, BUT NOT TESTOSTERONE, INCREASES PLATELET AGGREGATION MEDIATED BY ENDOTHELIAL CELLS

Yogyakarta, 1st-4th September 2015

IKHLAS MUHAMMAD JENIE[AB], BUDI MULYONO[B] SOEDJONO ASWIN[B], SRI KADARSIH SOEJONO[B]

[A]Faculty of Medicine & Health Sciences,
Universitas Muhammadiyah Yogyakarta

[B]Faculty of Medicine
Universitas Gadjah Mada Yogyakarta

BACKGROUND

- Endothelial cells (EC) form continues monolayer epithelial cells lining the internal lumen of the blood vessels.
- In normal physiological condition, EC have antithrombotic and antiplatelets properties.
  - Intact endothelium protects bood cells from contacted with extracellular matrix subendothelium
  - Normal EC synthesize and secrete thromboregulators, such as NO and prostacyclin
- In perturbed homeostasis, such as being exposed to hyperglycemia, EC may change to be pro thrombotic and pro platelet-activation, -adhesion, and -aggregation.

- Male sex has been recognized as a traditional risk factor in CVD for a long time ago.
- In recent years, testosterone (T) gets more attention as a potential player in cardiovascular diseases (CVD).
- However, the results of studies examining the relation between T and CVD are still conflicting.

- For example, as the level of serum T is rather low in patients with CVD, as well as in aging men, T replacement offers a potential benefit.
- Abrupt uprising of T level, such as experienced by athletes who take androgen, is associated with CV events.

- In CV events, platelet aggregation can be regarded as the early event that will lead to thrombosis formation
- EC is a non classical target organ for T, as it contains androgen receptor, estrogen receptor and enzymes that metabolize T, such as aromatase that catalyzes the conversion of T into E2

OBJECTIVE

- To examine whether exposure testosterone, high glucose, or combination of both to endothelial cells affects platelet aggregation

RESEARCH METHOD

In vitro experimental study

Endothelial cell culture derived from human umbilical vein (HUVEC)

2x4 factorial design

2X4 FACTORIAL DESIGN

	Normoglucose	High glucose
Without T	G1	G5
With T 1 nM	G2	G6
With T 10 nM	G3	G7
With T 100 nM	G4	G8

SAMPLE

Collection of umbilical cord from midwifery

Isolation of endothelial cells with enzymatic disaggregation

Primary culture of human umbilical vein endothelial cell (HUVEC)

TREATMENT

Monolayer subconfluency

Morphological & immunological identification

Harvesting & cell count

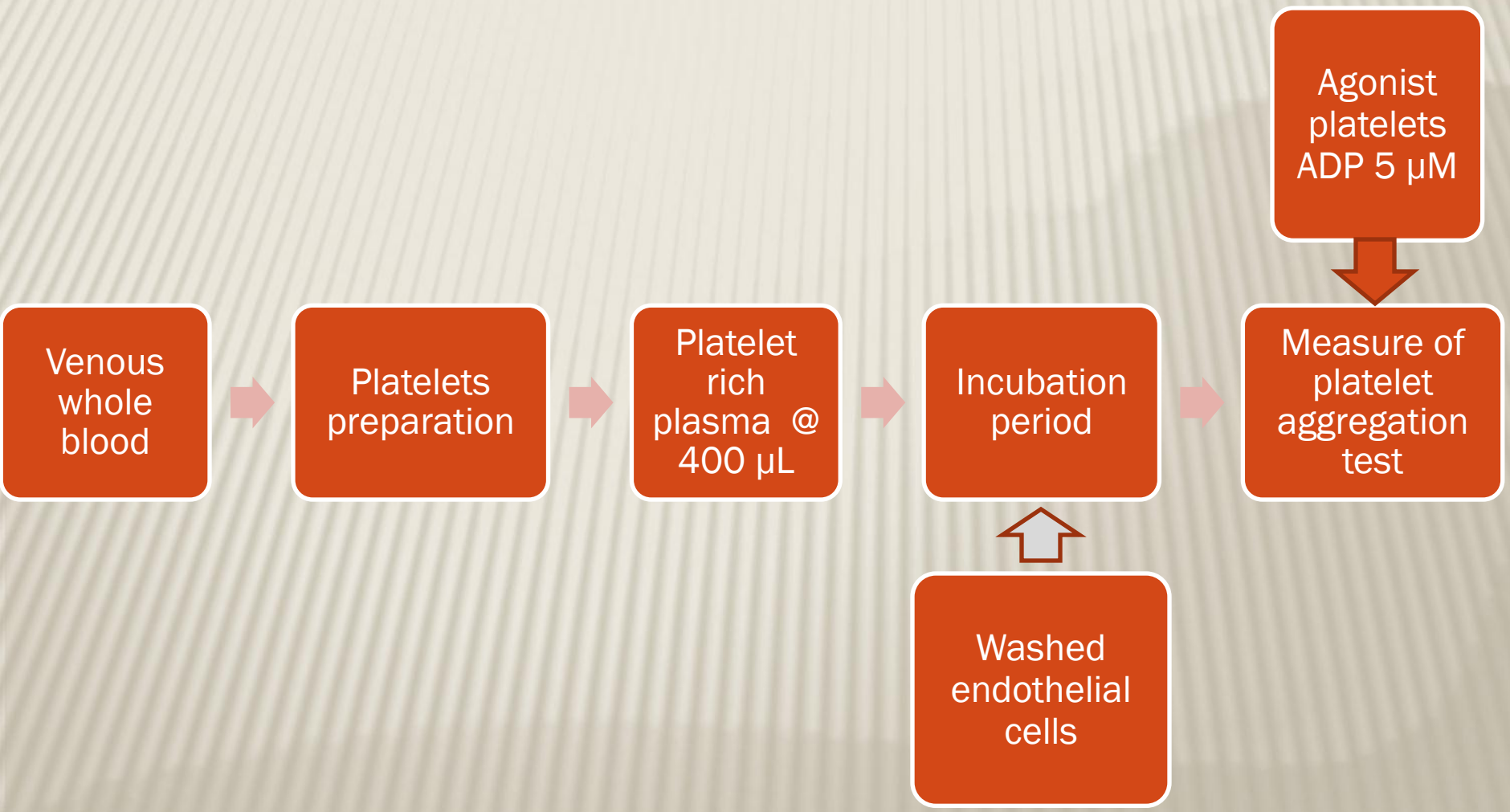
Subculturing (grouped)

TREATMENT

Exposure of testosterone & glucose medium for 24 hours

Washed (treatment ended) & harvesting

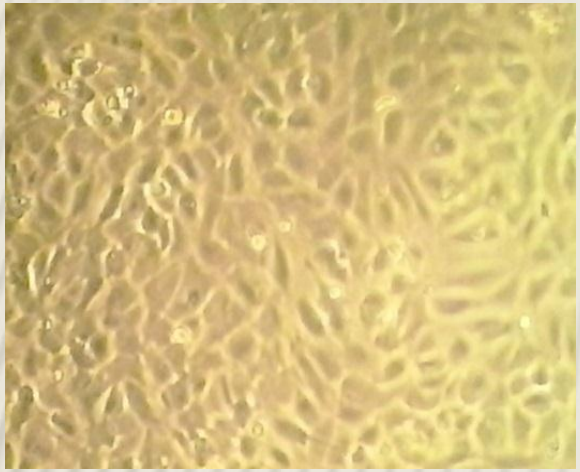
PLATELET AGGREGATION TEST



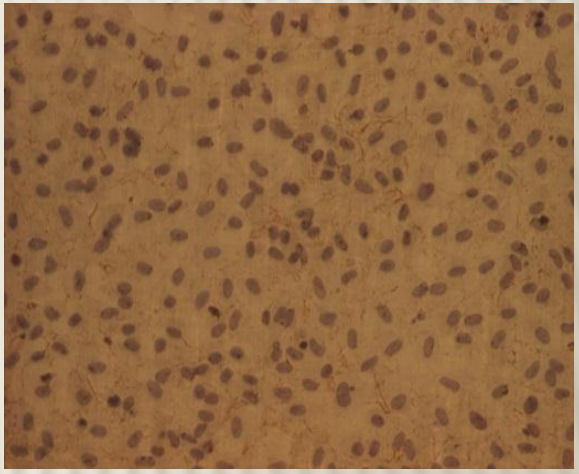
RESULTS

- Morphological and immunological identification of HUVEC
- Percentage of maximal platelet aggregation

PRIMARY HUVEC



Morphologi of HUVEC



Positive expression of von Willebrand factor

PLATELET AGGREGATION MEDIATED BY ENDOTHELIAL CELLS EXPOSED TO TESTOSTERONE & GLUCOSE MEDIUM

	Normoglucose	High glucose
Without T	29.3 ± 5.8	46.5 ± 10.7
With T 1 nM	25.0 ± 6.8	43.9 ± 1.4
With T 10 nM	28.5 ± 7.0	48.1 ± 0
With T 100 nM	29.1 ± 9.9	37.4 ± 2.3

DATA ANALYSIS

- One-way ANOVA analysis:
  - Exposure of T at various doses to EC did not show significant influences to maximal platelet aggregation in either NG ($p = 0.144$) or HG medium ($p = 0.916$)

DATA ANALYSIS

- Analysis of varians for 2x4 factorial design:
  - A main effect of glucose to EC to maximal platelet aggregation ($p = 0.004$)
  - No main effect of T to EC to maximal platelet aggregation ($p = 0.073$)
  - No interaction between T and glucose to EC to maximal platelet aggregation ($p = 0.69$)

DISCUSSION

This study found that:

- Exposure of testoterone to endothelial cells did not influence platelet aggregation
- Exposure of high glucose to endothelial cells increases platelet aggregation

CONTROVERCY REGARDING TESTOSTERONE EFFECT TO PLATELET AGGREGATION

Pro aggregatory

- Rosenblum et al. (1987)
  - - in vivo implant
- Matsuda et al. (1994)
  - - in vitro PRP directly
- Ajayi & Halushka (2005)
  - - in vitro PRP directly

Anti aggregatory

- Campello et al. (2012)
- Cutini et al. (20012)
  - - in vitro through endothelial cells culture from rat aortic ring

DISCUSSION

- Implanting androgen (T or DHT) pellets increased platelet aggregation in mice (Rosenblum et al., 1987)
- T increased the density of thromboxane receptors in platelets (Matsuda et al., 1994; Ajayi & Haluskha, 2005)

- The results of this study do not support Campelo et al. (2012) and Cutini et al. (2012) who reported the inhibition of platelet aggregation by T via EC by increasing NO release
- The differences, in part, may lie in sample used (EC)
  - This study used HUVEC, whereas Campelo et al. (2012) and Cutini et al. (2012) used RAEC

- HG condition in vitro mimics hyperglycemia in DM patients
- T2DM patients have exaggerated platelet reactivity (Vinik, 2001; Schneider, 2009)
- Diabetic environment promotes endothelial perturbation & dysfunction (Popov, 2010)

- Mechanism of ED in hyperglycemia:

  EC secrete prostanoid that functions as vasoconstrictor and pro platelet aggregation, i.e. thromboxane A_2, much more than prostanoid that functions as vasodilator and antiplatelet aggregation, i.e. prostacyclin (Cosentino et al., 2003).

Conclusion

Exposure of high glucose to endothelial cells increases platelet aggregation

Exposure of testosterone to endothelial cells did not influence platelet aggregation

THANK YOU VERY MUCH