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LAMINAR SHEAR STRESS PROMOTES ENDOTHELIAL CELL MIGRATION AND INHIBITS CELL APOPTOSIS IN THE PRESENCE OF HYDROXYUREA

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Abstract

Cell migration plays important roles in both physiological and pathological processes. Recent studies have shown that hydroxyurea, which is an anti-proliferative inhibitor, can affect cell morphology and specific gene expression of endothelial cells (ECs). *In vivo*, the functions of ECs are modulated by shear stress. It is well known that shear stress can have effects on EC migration by affecting cell morphology, cytoskeletal arrangement and cell-cell junction, and activating mechanosensors, inducing the changes of signaling pathways, and then increasing or decreasing the expression of gene and protein. However, the influences of hydroxyurea on EC function under shear stress are still unclear. In present study, we investigated the effects of hydroxyurea on EC proliferation, apoptosis and migration under laminar shear stress. The results showed that hydroxyurea prevented growth of ECs in a dose-dependent manner. Hydroxyurea at 2 mM completely inhibited the proliferation of ECs. The results also demonstrated that hydroxyurea induced EC apoptosis, but it was inhibited by 15.27 dyn/cm^2 laminar shear stress. Furthermore, shear stress induced cell migration in the presence of hydroxyurea. Therefore, 2 mM hydroxyurea, which completely inhibited the proliferation of HUVECs, could be used to eliminate any confounding effect of shear stress on proliferation in shear stress-induced cell migration. These results also do confirm that shear stress plays important roles in achieving and maintaining the stabilization of ECs.

Key words: HUVECs, shear stress, hydroxyurea, proliferation, apoptosis, migration.

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Abbreviations: ANOVA: analysis of variance; **DNA**: deoxyribonucleic acid; **dNTPs**: deoxyribonucleotide triphosphates; **ECs**: endothelial cells; **EdU**: 5-Ethynyl-2´-deoxyuridine ; **ET-1** : endothelin-1 ; **FN**: Fibronectin; **HUVECs**: human umbilical vein endothelial cells; **ICAM-1**: intercellular cell adhesion molecule-1; **IFNγ**: interferon-gamma; **TNFα**: tumor necrosis factor-alpha; **VCAM-1**: vascular cell adhesion molecule-1; **VLA-4**: very late activation antigen 4; **vWF**: von Willebrand factor.

INTRODUCTION

Hydroxyurea, which was first synthesized in Germany in 1869, has been known to have an anti-proliferative effect. It has long been used to treat a variety of malignant disorders, including myeloproliferative diseases, sickle cell anemia, ovarian carcinomas, and head and neck cancer (7, 40).

Hydroxyurea (5 mM) has been used in many previous studies to inhibit cell proliferation, such as human vascular smooth muscle cell migration under static conditions (41), human aortic endothelial cell migration under static conditions (20), irradiated human vascular smooth muscle cell migration (31), and irradiated human skin fibroblast cell migration (50). It is also documented that hydroxyurea at 2 mM completely inhibited human umbilical vein endothelial cells (HUVECs) proliferation on von Willebrand factor (vWF)/collagen and fibronectin (FN)/collagen (8), and had only a limited

inhibitory effect on endothelial cell (EC) migration under static conditions (26). Several studies have shown that desired effects of hydroxyurea are dependent on cell type, concentration, and stage of the cell cycle at the time of use (18, 38, 43). Induction of apoptosis may occur only in some types of cells, such as those of haematopoietic lineage (21). In addition to inhibiting ribonucleotide reductase, a recent investigation showed that hydroxyurea downregulated endothelin-1 (ET-1) and vascular cell adhesion molecule-1 (VCAM-1) gene expression and upregulated intercellular cell adhesion molecule-1 (ICAM-1) gene expression in cultured human bone marrow endothelial cells and EA.hy926 cells (6). ET-1 promotes migration of ECs (32, 34). ICAM-1 facilitates the development of cell polarity and modulates EC migration through a pathway regulating endothelial nitric-oxide synthase activation and organization of the actin cytoskeleton (23). The expression levels of these cell surface and soluble adhesion molecules (ICAM-1 and VCAM-1) can be increased by the proinflammatory cytokines (i.e., TNFα and IFNγ) (6). The effcts could be specifically blocked by antibodies to very late activation antigen 4 (VLA-4) and VCAM-1 (30). On the other hand, these cytokines don't have any effect on the proportion of cells in S-phase (6). It is suggested that cell cycle changes alone can't explain the changes in the expression of these molecules. Although the mechanism of action of hydroxyurea, either through its action on gene expression or on cell cycle or both, is still unclear, it is well known that hydroxyurea has influence on the morphology and function of ECs. For instance, hydroxyurea-treated vascular ECs showed significant morphologic changes, such as an increase in apparent cell size, accompanied by an increase in cell Na and K contents (1).

We and others have also shown that shear stress can modulate EC migration in blood vessels by affecting cell morphology (24), cytoskeletal arrangement (11), cell-cell junction (28, 33), and activating mechanosensors, signaling pathways, and gene and protein expressions (13, 49). ET-1 was shown to be dramatically reduced by exposure to 25 dyn/cm^2 shear flow (44). The expression of VCAM-1 and ICAM-1 was shear stress-dependent (47). Thus, both of shear stress and hydroxyurea may affect the expression of ET-1, VCAM-1 and ICAM-1. The potential influence of hydroxyurea on cell migration and its interplay with mechanical force must be considered. The present study focuses on the proliferation, the apoptosis and the migration of hydroxyurea-treated HUVECs when exposed to 15.27 dyn/cm^2 laminar shear stress.

MATERIALS AND METHODS

Cell culture

Human umbilical vein endothelial cells (HUVECs) were isolated from human umbilical cord vein with 0.1% Collagenase II (Sigma, St. Louis, MO) and maintained in M-199 medium supplemented with 20% fetal calf serum, 2 mM L-glutamine, 100 μg/ml streptomycin, 100 U/ml penicillin and 20 μg/ml endothelial cell growth supplement (Millipore Co., Billerica, MA). Cells were seeded onto a glass slide that had been pre-coated with FN (50 μg/ml) and all cell cultures were grown in a humidified 5%/95% CO_2/air incubator at 37°C. Confluent cultures of HUVECs exhibited the typical cobblestone morphology, and most of those cells contained factor VIII-related antigen and Weibel–Palade body.

Effects of hydroxyurea on EC proliferation and apoptosis under laminar shear stress

The shear stress experiments were performed as previously described (10, 19, 49). In brief, 15.27 dyn/cm^2 laminar shear flow were applied while HUVECs were seeded on glass chamber slides and cultured until confluence. The circulation fluid was serum-free M-199 medium with 1 mM and 2 mM hydroxyurea, respectively. The flow system was kept at 37°C and ventilated with 95% humidified air with 5% CO_2. Shear controls were concurrently performed by using the circulation fluid M-199 medium without hydroxyurea. Static controls were also concurrently performed.

After exposure to laminar shear stress for 2 hr, cell proliferation was detected by using the Cell-Light^{TM} EdU DNA cell proliferation assay kits (RIBOBIO, Guangzhou, China) according to the manufacturer's instructions. Nuclei were also stained with the blue-fluorescent counterstain Hoechst 33342. The condensed chromatin in apoptotic cells was stained more brightly than the chromatin in normal cells. Images were captured by a fluorescence microscope (Nikon, TE 2000, Japan). The proliferative activity index was defined as the percentage of S-phase cells. The apoptosis index was defined as the percentage of apoptosis cells in all cells.

Scratch wound migration assay

Cell migration was measured using a monolayer scratch injury assay, as described previously (17, 29, 49). Briefly, the HUVECs were seeded on glass chamber slides and cultured until confluence. Then, HUVECs were pretreated for 2 hr with 2 mM hydroxyurea before exposed to shear stress, but the control cells were not treated with hydroxyurea. A uniform straight scratch was performed in the cell monolayer by using a plastic Cell Scrapper (Corning Inc., Corning, NY, USA). Monolayers were washed gently, marked (for reference) and photographed using an inverted microscope (Model CKX41SF, Olympus Optical Co. Ltd., Tokyo, Japan) before shear stress experiments.

Effect of hydroxyurea on EC migration under laminar shear stress

Laminar shear flows were applied in the direction orthogonal to the wound. The circulation fluid was serum-free M-199 medium with 2 mM hydroxyurea. The flow system was kept at 37°C and ventilated with 95%

humidified air with 5% CO_2. 15.27 dyn/cm^2 shear stress was imposed in this study. Images of the wounds were acquired after exposure to shear stress for 2 hr. Shear controls were concurrently performed by using the circulation fluid M-199 medium without hydroxyurea. Static controls were also concurrently performed. Cells that had migrated into monolayer wounds were counted. Then, the percentages of migrated cells were calculated by dividing the total cells with migrated cells.

Assessment of wound closure

The wound closure was assessed as our previous described (49). Briefly, HUVEC migration during the process of wound closure was analyzed using the Image Pro Plus 6.0 image analysis software (Media Cybernetics, Inc., Bethesda, MD). The acquired image was converted from pixels to micrometers with the use of a calibration image, then the area could be tabulated while an outline of the wound was traced (4, 42). The levels of wound closure could be assessed by the ratio of closure area to initial wound as follows:

$$R_n = \frac{(A_0 - A_n)}{A_0}$$

where R_n represents the percentage of wound closure; A_n represents the residual area of wound at the metering point n (h) and A_0 represents the area of initial wound.

Statistical analysis

Data were presented as means±SD at least 10 images obtained from three different experiments, unless otherwise indicated. Statistical analysis was performed by the one-way ANOVA test using SPSS 17.0 software package. Differences in means were considered significant if $P<0.05$.

RESULTS

Cell proliferation and apoptosis

Cell proliferation was determined by the EdU detection with red-fluorescent dye (Apollo®) (Fig. 1A). The proliferative activity index was defined as the percentage of S-phase ECs (Fig. 1B). After exposure to shear stress for 2 hr, the addition of 2 mM hydroxyurea completely inhibited the EC proliferation (Fig. 1A). In contrast, no significant inhibition of proliferation occurred at a dose of 1 mM hydroxyurea (Fig. 1A and B).

Nuclei were also stained with the blue-fluorescent counterstain Hoechst 33342. The condensed chromatin in apoptotic cells was stained more brightly than the chromatin in normal cells (Fig.1 A). The apoptosis of ECs was greatly enhanced by 1 mM and 2 mM hydroxyurea under static conditions (Fig. 1A). Specifically, the apoptosis index was defined as the percentage of apoptosis ECs in total cells (Fig. 1C). Under static conditions, 1 mM and 2 mM hydroxyurea induced significant increase of the EC apoptosis index compared with 0 mM hydroxyurea (0 mM hydroxyurea, 7.2%±2.3%; 1 mM hydroxyurea, 17.3%±3.6%; 2 mM hydroxyurea, 26.7%±4.3% Fig. 1C). Specially, 15.27 dyn/cm^2 shear stress can inhibit hydroxyurea-induced EC apoptosis (Fig. 1A and C). The apoptosis indexes of hydroxyurea-treated EC under shear stress were decreased to nearly the non-treated cells under static conditions. It was suggested that shear stress could inhibit the hydroxyurea-induced EC apoptosis. Taken together, hydroxyurea had no significant effects on EC apoptosis under shear stress, suggesting 2 mM hydroxyurea was able to inhibit proliferation through its cytostatic effect without damaging the viability of HUVECs under shear stress.

Cell migration and wound closure

The hydroxyurea-treated monolayers showed a decrease in wound closure under both static and shear stress conditions (Figs. 2 and 3). Shear stress enhanced the wound closure (40.67±2.90 under shear stress vs. 19.19±6.59 under static conditions, $P<0.001$). While cells treated with 2 mM hydroxyurea, the wound closure under shear stress was also larger than that under static conditions (21.70±0.94 under shear stress vs. 10.53±0.30 under static conditions, $P<0.001$).

Cells that had migrated into monolayer wounds were counted (Fig. 3). Under static conditions (Fig. 2A and C), fewer hydroxyurea-treated cells migrated into monolayer wounds, as compared with untreated cells (hydroxyurea-treated cells, 6.00±2.00 vs. non-treated cells, 29.67±2.08, $P<0.001$ Fig. 3). The similar phenomena were obtained under shear stress conditions (Fig. 2B and D), the number of hydroxyurea-treated cells was less than that of untreated cells (hydroxyurea-treated cells, 14.67±1.53 vs. non-treated cells, 55.00±2.65, $P<0.001$ Fig. 3).

Interestingly, the percentage of migrated hydroxyurea-treated cells has no significantly difference with that of migrated non-treated cells at the same conditions (shear stress or static condition, $P>0.05$ Fig. 3). But the percentage under shear stress was greater than that under static condition. It was indicated that hydroxyurea affected wound closure and migrated cell number under shear stress due to its anti-proliferative effect. Taken together, these results suggested that shear stress-induced wound closure was primary due to cell migration in the presence of hydroxyurea.

Furthermore, hydroxyurea-treated cells under shear stress of 15.27 dyn/cm^2 showed more cells migrated into monolayer wounds than that under static conditions. Specifically, compared

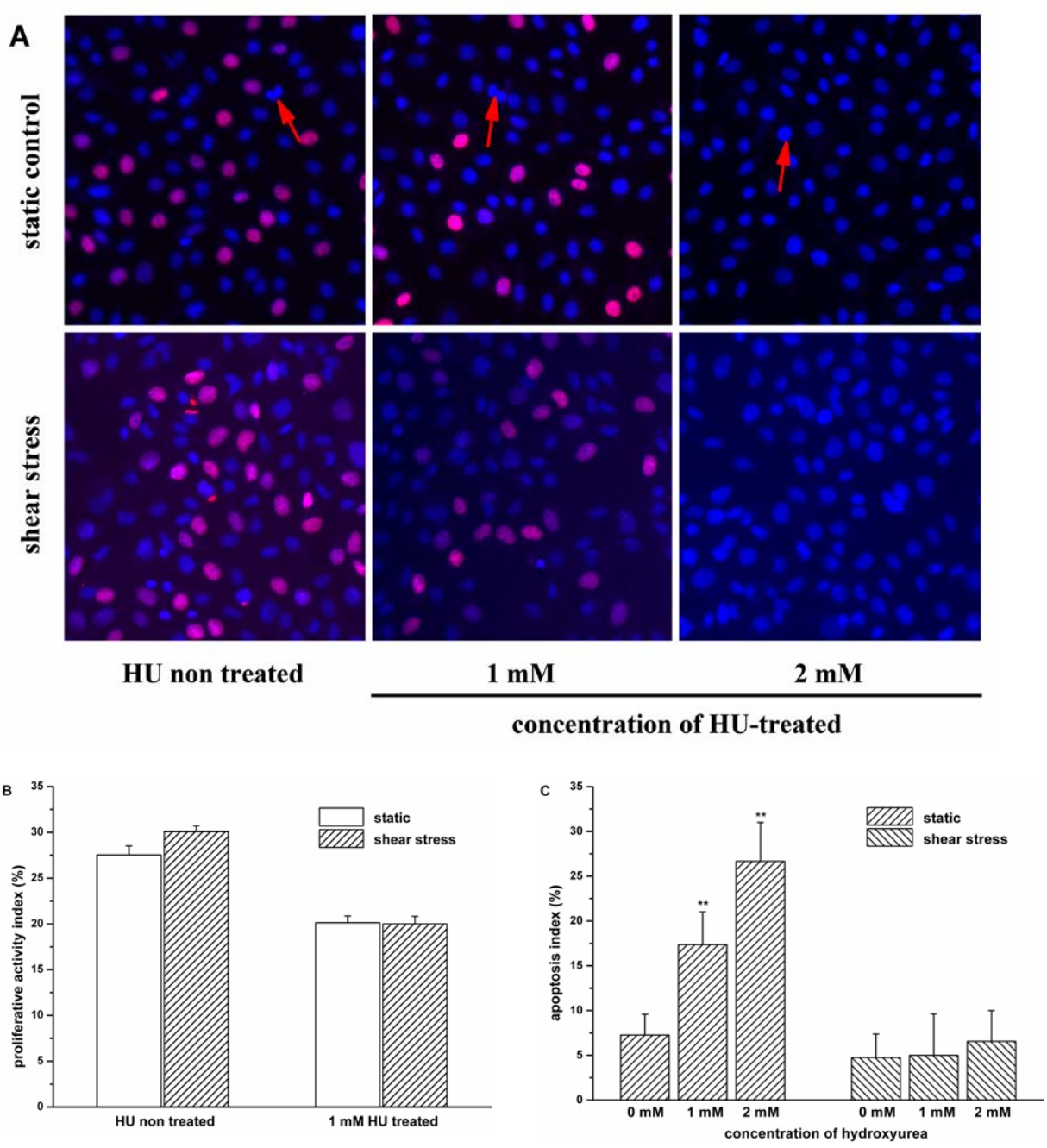

Figure 1. Effects of hydroxyurea (HU) on the proliferation and the apoptosis of endothelial cells (ECs). (A) The proliferation and the apoptosis of ECs under 15.27 dyn/cm^2; (B) The proliferative activity of hydroxyurea-treated ECs; (C) The apoptosis of ECs after treated with hydroxyurea. After exposure to laminar shear stress for 2 hr, cell proliferation was determined by the EdU detection with red-fluorescent dye (Apollo®). Nuclei were also stained with the blue-fluorescent counterstain Hoechst 33342. The condensed chromatin in apoptotic cells was stained more brightly than the chromatin in normal cells. The proliferative activity index was defined as the percentage of S-phase cells. The apoptosis index was defined as the percentage of apoptosis cells. ***P*<0.01 vs. 0 mM hydroxyurea under static conditions. Arrows indicated the apoptotic nuclei.

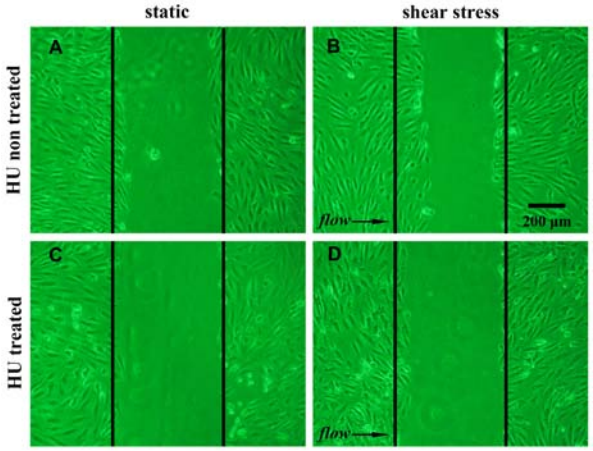

Figure 2. Morphology of wounded ECs after exposure to 15.27 dyn/cm^2 for 2 hr. (A) Cells were treated without hydroxyurea (HU-non treated) under static conditions; (B) Cells were HU-non treated under laminar shear stress conditions; (C) Cells were treated with 2 mM hydroxyurea (HU-treated) under static conditions; (D) Cells were HU-treated under laminar shear stress conditions. Arrow indicated the direction of flow.

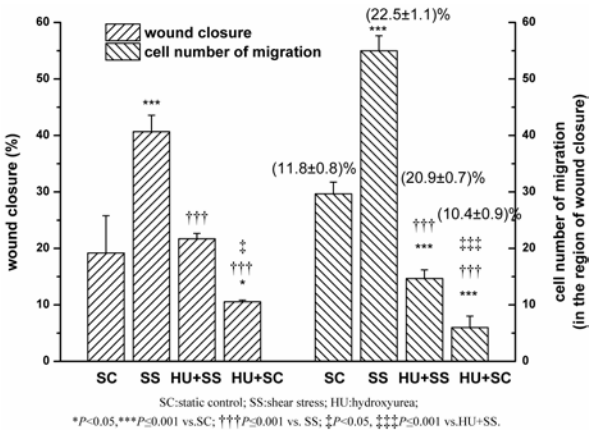

Figure 3. Effects of 2 mM hydroxyurea on EC migration after exposure to 15.27 dyn/cm^2 for 2 hr. Both the percentage of wound closure and the number of cells migrated into the wound were obtained. The percentages of migrated cells were also labeled.

with the static conditions, the hydroxyurea-treated cells' wound closure and the percentage of migrated cells under shear stress was respectively increased about 9% and 11%. Therefore, shear stress promotes wound closure and EC migration in the presence of hydroxyurea.

DISCUSSION

Our present investigation demonstrated that 2 mM hydroxyurea completely inhibited the proliferation of human umbilical vein endothelial cells (HUVECs), and enhanced EC apoptosis. But its effect on cell apoptosis was inhibited by 15.27 dyn/cm^2 laminar shear stress. The results also showed that shear stress promoted EC migration in the presence of hydroxyurea. These findings suggest that shear stress significantly contributes to EC migration and integrity by inhibition of programmed cell death.

Several investigations have been showed that hydroxyurea at 2 mM completely inhibited HUVEC proliferation on vWF/collagen and FN/collagen, and had only a limited inhibitory effect on EC migration under static conditions. Additionally, hydroxyurea has been shown anti-proliferative and cytotoxic effects on HeLa cells in a dose-dependent manner (2). The response of HeLa cells to hydroxyurea is based on the growth phase of those cells. When non-confluent (proliferating) cultures of HeLa cells were treated with a 100 ng/ml concentration of hydroxyurea, a significant anti-proliferative effect was noted. However, when confluent (non-proliferating) HeLa cells were treated with the same concentration, only 20% cell death was observed. It was suggested that hydroxyurea mainly interfered with the growth of proliferating cells, and that hydroxyurea had direct cytotoxic effects on non-proliferating HeLa cells. Our results showed that 1 and 2 mM hydroxyurea enhanced the confluent HUVECs apoptosis while 2 mM hydroxyurea inhibited cell proliferation. Linke et al. (27) suggested that hydroxyurea, which was dependent on concentration, through a p53-dependent effect, lead to reversible cell quiescence (arrest above G1), was most probably due to depletion of deoxyribonucleotide triphosphates (dNTPs) and senescence-like irreversible cell arrest.

Shear stress plays important roles in EC apoptosis. Several studies reported that shear stress could inhibit the proliferation and apoptosis of HUVECs (3, 16, 25), the lack of shear stress triggers apoptosis in ECs (16, 22). Exposure of HUVEC to laminar flow (15 dyn/cm^2) completely abrogated apoptosis induced by TNFa and reduced DNA fragmentation even below baseline levels, whereas 5 dyn/cm^2 was less effective (16). This effect could be mediated by a mechanism independent of DNA synthesis. Akimoto et al. (3) showed that laminar shear stress (5 and 30 dyn/cm^2) induced cell cycle arrest by upregulating p21. Our results also showed shear stress could protect ECs from apoptosis induced by hydroxyurea; 2 mM hydroxyurea completely inhibited EC proliferation, and 15.27 dyn/cm^2 inhibited the hydroxyurea-induced EC apoptosis.

Considering the number of migrated cells and the degree of wound closure might be increased through inhibiting the apoptosis, we calculated the percentage of migrated cells. The results showed that the percentage of migrated hydroxyurea-treated cells had no significantly difference with that of migrated non-treated cells at the same conditions (shear stress or static condition). It was indicated that hydroxyurea affected wound closure and migrated cell number under shear stress due to its anti-proliferative effect. Thus, the rate of wound closure decreased in hydroxyurea-treated cells may due to the reduction of cell number but was not results of the cell activity or cell apoptosis change.

Shear stress acts as an endothelial cell "survival" factor (45). In areas downstream of vessel bifurcations, laminar shear stress predominates, and the endothelial cells experience pulsatile flow, with shear stress on the order of 10 to 30 dyn/cm^2 (45). These physiological levels of shear stress provide a protective role for the functional integrity of ECs (35, 36). Further, the endothelium in these regions maintains circulatory and blood vessel integrity through its ability to regulate several different processes: coagulation, growth of underlying smooth muscle, transmigration of leukocytes, and lipoprotein uptake and metabolism (45). Injury of the vascular endothelium is an early critical event in the pathogenesis of atherosclerosis (39). Changes in local artery geometry during atherogenesis further modify the shear stress characteristics at the endothelium. Then, shear stress spans a range of spatiotemporal scales and contributes to regional and focal heterogeneity of endothelial gene expression that leads to cardiovascular disease (15). Shear stress has direct influences on the pathogenesis of atherosclerosis via regulation of endothelial cell function and integrity. For instance, endothelial cells on the border of a wound edge fails to maintain contact with neighboring cells and are oriented randomly under low shear stress (46). In the branch regions of the arterial trees, flow with low magnitude (14), none of a net forward direction (12), may result in the ECs dysfunction (5, 37). The DNA synthesis of ECs preferentially occurs at these regions, where atherosclerosis is initiated (48). Several investigations have indicated that increased cell division may accelerate endothelial permeability (9). Stabilization of ECs may be achieved and maintained by restricting EC proliferation and apoptosis, and by accelerating EC migration, and thereby inhibit damage to the arterial vascular wall. 15.27 dyn/cm^2 laminar shear stress inhibits cell apoptosis and promotes cell migration in the presence of hydroxyurea. It does confirm the important roles of shear stress in the stabilization of ECs and the endothelium integrity.

Finally, 2 mM hydroxyurea, which can completely inhibit the proliferation of HUVECs, could be used to eliminate any confounding effect of shear stress on proliferation in shear stress-induced cell migration. However, the mechanism, involved in the shear stress-induced EC migration in the presence of hydroxyurea, remains to be further investigated. Furthermore, clarify the molecular mechanism underling these results may provide novel molecular targets to associate with hydroxyurea therapy *in vivo*.

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REFERENCES

1. Adragna, N.C., Fonseca, P. and Lauf, P.K., Hydroxyurea affects cell morphology, cation transport, and red blood cell adhesion in cultured vascular endothelial cells. *Blood* 1994, **83**: 553-560.

2. AKAÇ, H. and ÖZES, O.N., Hydroxyurea induces p53 accumulation and apoptosis in human carcinoma cells. *Turk. J. Biol*. 2002, **26**: 145-150.

3. Akimoto, S., Mitsumata, M., Sasaguri, T. and Yoshida, Y., Laminar shear stress inhibits vascular endothelial cell proliferation by inducing cyclin-dependent kinase inhibitor p21(Sdi1/Cip1/Waf1). *Circ. Res.* 2000, **86**: 185-190.

4. Albuquerque, M.L., Waters, C.M., Savla, U., Schnaper, H.W. and Flozak, A.S., Shear stress enhances human endothelial cell wound closure in vitro. *Am. J. Physiol. Heart Circ. Physiol*. 2000, **279**: H293-302.

5. Barakat, A. and Lieu, D., Differential responsiveness of vascular endothelial cells to different types of fluid mechanical shear stress. *Cell Biochem. Biophys.* 2003, **38**: 323-343.

6. Brun, M., Bourdoulous, S., Couraud, P.O., Elion, J., Krishnamoorthy, R. and Lapoumeroulie, C., Hydroxyurea downregulates endothelin-1 gene expression and upregulates ICAM-1 gene expression in cultured human endothelial cells. *Pharmacogenomics J.* 2003, **3**: 215-226.

7. Buck, S.B., Bradford, J., Gee, K.R., Agnew, B.J., Clarke, S.T. and Salic, A., Detection of S-phase cell cycle progression using 5-ethynyl-2'-deoxyuridine incorporation with click chemistry, an alternative to using 5-bromo-2'-deoxyuridine antibodies. *Biotechniques* 2008, **44**: 927-929.

8. Cai, G., Lian, J., Shapiro, S.S. and Beacham, D.A., Evaluation of endothelial cell migration with a novel in vitro assay system. *Methods Cell Sci* 2000, **22**: 107-114.

9. Caplan, B.A. and Schwartz, C.J., Increased endothelial cell turnover in areas of in vivo Evans Blue uptake in the pig aorta. *Atherosclerosis* 1973, **17**: 401-417.

10. Cheng, M., Liu, X., Li, Y., Tang, R., Zhang, W., Wu, J., Li, L., Gang, Y. and Chen, H., IL-8 gene induction by low shear stress: pharmacological evaluation of the role of signaling molecules. *Biorheology* 2007, **44**: 349-360.
11. Cheng, M., Wu, J., Liu, X., Li, Y., Nie, Y., Li, L. and Chen, H., Low shear stress-induced interleukin-8 mRNA expression in endothelial cells is mechanotransduced by integrins and the cytoskeleton. *Endothelium* 2007, **14**: 265-273.
12. Chien, S., Mechanotransduction and endothelial cell homeostasis: the wisdom of the cell. *Am. J. Physiol. Heart Circ. Physiol.* 2007, **292**: H1209-1224.
13. Chien, S., Li, S. and Shyy, Y.J., Effects of mechanical forces on signal transduction and gene expression in endothelial cells. *Hypertension* 1998, **31**: 162-169.
14.Davies, P.F., Flow-mediated endothelial mechanotransduction. *Physiol. Rev.* 1995, **75**: 519-560.
15. Davies, P.F., Hemodynamic shear stress and the endothelium in cardiovascular pathophysiology. *Nat. Clin. Pract. Cardiovasc. Med.* 2009, **6**: 16-26.
16. Dimmeler, S., Haendeler, J., Rippmann, V., Nehls, M. and Zeiher, A.M., Shear stress inhibits apoptosis of human endothelial cells. *FEBS Lett.* 1996, **399**: 71-74.
17. Fan, W.H., Pech, M. and Karnovsky, M.J., Connective tissue growth factor (CTGF) stimulates vascular smooth muscle cell growth and migration in vitro. *Eur. J. Cell Biol.* 2000, **79**: 915-923.
18. Fibach, E., Burke, L.P., Schechter, A.N., Noguchi, C.T. and Rodgers, G.P., Hydroxyurea increases fetal hemoglobin in cultured erythroid cells derived from normal individuals and patients with sickle cell anemia or beta-thalassemia. *Blood* 1993, **81**: 1630-1635.
19. Gojova, A. and Barakat, A.I., Vascular endothelial wound closure under shear stress: role of membrane fluidity and flow-sensitive ion channels. *J. Appl. Physiol.* 2005, **98**: 2355-2362.
20. Hamuro, M., Polan, J., Natarajan, M. and Mohan, S., High glucose induced nuclear factor kappa B mediated inhibition of endothelial cell migration. *Atherosclerosis* 2002, **162**: 277-287.
21. Hayashi, M., Hamasu, T., Endoh, D., Shimojima, R. and Okui, T., Inhibition of replication induces non-apoptotic cell death in fibroblast cell lines derived from LEC rats. *J. Vet. Med. Sci.* 2003, **65**: 249-254.
22. Kaiser, D., Freyberg, M.A. and Friedl, P., Lack of hemodynamic forces triggers apoptosis in vascular endothelial cells. *Biochem. Biophys. Res. Commun.* 1997, **231**: 586-590.
23. Kevil, C.G., Orr, A.W., Langston, W., Mickett, K., Murphy-Ullrich, J., Patel, R.P., Kucik, D.F. and Bullard, D.C., Intercellular adhesion molecule-1 (ICAM-1) regulates endothelial cell motility through a nitric oxide-dependent pathway. *J. Biol. Chem.* 2004, **279**: 19230-19238.
24. Levesque, M.J. and Nerem, R.M., The elongation and orientation of cultured endothelial cells in response to shear stress. *J. Biomech. Eng.* 1985, **107**: 341-347.
25. Levesque, M.J., Nerem, R.M. and Sprague, E.A., Vascular endothelial cell proliferation in culture and the influence of flow. *Biomaterials* 1990, **11**: 702-707.
26. Lian, J., Guoping, C., Shapiro, S.S., Tran, L.P. and Beacham, D.A., Glycoprotein Ibalpha can mediate endothelial cell migration on von Willebrand factor-containing substrata. *Exp. Cell Res.* 1999, **252**: 114-122.
27. Linke, S.P., Clarkin, K.C., Di Leonardo, A., Tsou, A. and Wahl, G.M., A reversible, p53-dependent G0/G1 cell cycle arrest induced by ribonucleotide depletion in the absence of detectable DNA damage. *Genes Dev.* 1996, **10**: 934-947.
28. Liu, S.Q., Yen, M. and Fung, Y.C., On measuring the third dimension of cultured endothelial cells in shear flow. *Proc. Natl. Acad. Sci. U. S. A.* 1994, **91**: 8782-8786.
29. Liu, X., Luo, F., Pan, K., Wu, W. and Chen, H., High glucose upregulates connective tissue growth factor expression in human vascular smooth muscle cells. *BMC Cell Biol.* 2007, **8**: 1.
30. Lutty, G.A., Taomoto, M., Cao, J., McLeod, D.S., Vanderslice, P., McIntyre, B.W., Fabry, M.E. and Nagel, R.L., Inhibition of TNF-alpha-induced sickle RBC retention in retina by a VLA-4 antagonist. *Invest. Ophthalmol. Vis. Sci.* 2001, **42**: 1349-1355.
31. Majack, R.A. and Clowes, A.W., Inhibition of vascular smooth muscle cell migration by heparin-like glycosaminoglycans. *J. Cell. Physiol.* 1984, **118**: 253-256.
32. Malek, A.M., Zhang, J., Jiang, J., Alper, S.L. and Izumo, S., Endothelin-1 gene suppression by shear stress: pharmacological evaluation of the role of tyrosine kinase, intracellular calcium, cytoskeleton, and mechanosensitive channels. *J. Mol. Cell. Cardiol.* 1999, **31**: 387-399.
33. Melchior, B. and Frangos, J.A., Shear-induced endothelial cell-cell junction inclination. *Am. J. Physiol., Cell Physiol.* 2010, **299**: C621-629.
34. Milan, J., Charalambous, C., Elhag, R., Chen, T.C., Li, W., Guan, S., Hofman, F.M. and Zidovetzki, R., Multiple signaling pathways are involved in endothelin-1-induced brain endothelial cell migration. *Am. J. Physiol., Cell Physiol.* 2006, **291**: C155-164.
35. Nerem, R.M., Hemodynamics and the vascular endothelium. *J. Biomech. Eng.* 1993, **115**: 510-514.
36. Nerem, R.M., Harrison, D.G., Taylor, W.R. and Alexander, R.W., Hemodynamics and vascular endothelial biology. *J. Cardiovasc. Pharmacol.* 1993, **21**: S6-10.
37. Resnick, N., Yahav, H., Shay-Salit, A., Shushy, M., Schubert, S., Zilberman, L.C. and Wofovitz, E., Fluid shear stress and the vascular endothelium: for better and for worse. *Prog. Biophys. Mol. Biol.* 2003, **81**: 177-199.
38. Rocha, B., Larsson, E.L. and Freitas, A.A., Effects of hydroxyurea on concanavalin-A-induced T-cell proliferation. Depletion of T-cell growth factor-reactive and -producing T lymphocytes. *Scand. J. Immunol.* 1984, **19**: 315-321.
39. Ross, R., Cell biology of atherosclerosis. *Annu. Rev. Physiol.* 1995, **57**: 791-804.
40. Salic, A. and Mitchison, T.J., A chemical method for fast and sensitive detection of DNA synthesis in vivo. *Proc. Natl. Acad. Sci. U. S. A.* 2008, **105**: 2415-2420.
41. Sarkar, R., Meinberg, E.G., Stanley, J.C., Gordon, D. and Webb, R.C., Nitric oxide reversibly inhibits the migration of cultured vascular smooth muscle cells. *Circ. Res.* 1996, **78**: 225-230.
42. Savla, U. and Waters, C.M., Mechanical strain inhibits repair of airway epithelium in vitro. *Am. J. Physiol.* 1998, **274**: L883-892.
43. Schrell, U.M., Rittig, M.G., Anders, M., Kiesewetter, F., Marschalek, R., Koch, U.H. and Fahlbusch, R., Hydroxyurea for treatment of unresectable and recurrent meningiomas. I. Inhibition of primary human meningioma cells in culture and in meningioma transplants by induction of the apoptotic pathway. *J. Neurosurg.* 1997, **86**: 845-852.
44. Sharefkin, J.B., Diamond, S.L., Eskin, S.G., McIntire, L.V. and Dieffenbach, C.W., Fluid flow decreases preproendothelin mRNA levels and suppresses endothelin-1 peptide release in cultured human endothelial cells. *J. Vasc. Surg.* 1991, **14**: 1-9.
45. Traub, O. and Berk, B.C., Laminar shear stress: mechanisms by which endothelial cells transduce an

atheroprotective force. *Arterioscler. Thromb. Vasc. Biol.* 1998, **18**: 677-685.
46. Vyalov, S., Langille, B.L. and Gotlieb, A.I., Decreased blood flow rate disrupts endothelial repair in vivo. *Am. J. Pathol.* 1996, **149**: 2107-2118.
47. Walpola, P.L., Gotlieb, A.I., Cybulsky, M.I. and Langille, B.L., Expression of ICAM-1 and VCAM-1 and monocyte adherence in arteries exposed to altered shear stress. *Arterioscler. Thromb. Vasc. Biol.* 1995, **15**: 2-10.
48. Wright, H.P., Mitosis patterns in aortic endothelium. *Atherosclerosis* 1972, **15**: 93-100.
49. Zeng, Y., Sun, H.R., Yu, C., Lai, Y., Liu, X.J., Wu, J., Chen, H.Q. and Liu, X.H., CXCR1 and CXCR2 are novel mechano-sensors mediating laminar shear stress-induced endothelial cell migration. *Cytokine* 2011, **53**: 42-51.
50. Zungu, I.L., Mbene, A.B., Hawkins Evans, D.H., Houreld, N.N. and Abrahamse, H., Phototherapy promotes cell migration in the presence of hydroxyurea. *Lasers Med Sci* 2009, **24**: 144-150.

