Vortex-mediated Mechanical Stress Induces Integrin-dependent Cell Adhesion Mediated by Inositol 1,4,5-Trisphosphate-sensitive Ca\(^{2+}\) Release in THP-1 Cells\(^*\)

Received for publication, December 4, 2002, and in revised form, January 7, 2003 Published, JBC Papers in Press, January 7, 2003, DOI 10.1074/jbc.M212316200

Noboru Ashida\(\dagger\), Hajime Takechi\(\dagger\), Toru Kita\(\dagger\), and Hidenori Arai\(\dagger\)**

From the Department of Geriatric and Cardiovascular Medicine, Kyoto University Graduate School of Medicine, Kyoto, 606-8507, Japan

The nature of blood flow patterns and shear forces within blood vessels may be very variable depending upon vessel size, shape, branching, and partial obstructions (1). Biomechanical forces induced within the cardiovascular system affect gene expression in cells of blood vessel walls (2, 3) and functions of the cells in the vessel wall and in the fluid phase (4–8). Changes of shear forces occur in bifurcated or stenotic regions where atherosclerotic regions are prone to develop.

According to the multistep theory in cell transmigration, monocytes roll on the endothelial cells, interact with selectins, adhere to the endothelial cells by firm adhesion to ICAM-1\(^6\) and vascular cell adhesion molecule-1 (VCAM-1), and then migrate into the subendothelium (9). Rolling of monocytes on endothelial cells is dependent on the binding of E-selectin and sialyl Lewis X, and adhesion to the endothelium is dependent on the interaction between integrins on monocytes and adhesion molecules on the endothelial cells, such as VCAM-1 and ICAM-1. Integrins consist of several subtypes, and each subtype is specific for each ligand. For example, \(\alpha_\text{v}\beta_1\) integrin, VLA-4, binds to VCAM-1, and \(\beta_2\) integrins bind to ICAM-1. Fibronectin, one of the extracellular matrix proteins, is also known to bind to \(\beta_1\) integrins, mainly to \(\alpha_\text{v}\beta_1\) integrin. Thus activation of adhesion molecules in endothelial cells and leukocytes is important for the cell migration process.

In this study, we hypothesized that leukocyte adhesion might be increased at bifurcations and in the downstream of the restricted vessels. In normal laminar flow, it has been reported that human leukocytes respond to fluid shear stress by retracting pseudopods and down-regulation of integrins (10, 11), which is a requirement for normal passage of circulating leukocytes through the microcirculation. In the downstream of the region where the vessel lumen is partially occluded, however, a backward vortex can be observed where cells in the fluid phase are subjected to a vortex motion under low shear forces (1, 12–14). Although such a change of shear force on endothelial cells can regulate the expression of adhesion molecules resulting in the progression of atherosclerosis (15, 16), the effect of vortex-mediated mechanical stress on leukocytes has not yet been determined. If vortex-induced mechanical stress can induce cell adhesion in leukocytes, leukocytes would be more prone to attach to the endothelial lining in the turbulent flow because the residence time of leukocytes in the regions with nonlaminar flow is longer than in those with laminar flow (12, 13).

A variety of signaling systems are induced by a mechanosensor in endothelial cells. As a mechanosensor, stretch-activated channels have been reported to regulate Ca\(^{2+}\) influx induced by flow stress in cells such as endothelial cells or smooth muscle cells (17). There is much evidence that stretch increases intracellular Ca\(^{2+}\) levels (4, 17). Thus the importance of Ca\(^{2+}\) signaling in endothelial mechanotransduction has been established. However, the role of Ca\(^{2+}\) in cell response to the mechanical stress in leukocytes has not been examined so far. Therefore, the aim of this study was to examine the effect of...
mechanical stress on integrin-dependent cell adhesion in human monocyted THP-1 cells and to elucidate the role of Ca^{2+} signaling involved in this process.

**EXPERIMENTAL PROCEDURES**

**Reagents—**RPMI medium was obtained from Nissui Pharmaceuticals Co. Ltd. (Tokyo, Japan). Fetal calf serum was purchased from Grand Cayman (British West Indies). L-glutamine and penicillin/strep-tomyycin were obtained from Bio Whittaker (Walkersville, MD). Recombinant human soluble VCAM-1 and ICAM-1 were from Genzyme/Technie (Minneapolis, MN). Fibronectin, thapsigargin, W-7, ryanodine, U-73122, bovine serum albumin, RGDS peptides, and RGES peptides were from Sigma. Anti-human α4 (VLA-4) antibody was from Upstate Biotechnology (Lake Placid, NY). GdCl3•6H2O and NiCl2•6H2O were from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). BAPTA-AM was from Dojindo (Kumamoto, Japan).

**Cell Lines—**The monocytic cell line THP-1 was a generous gift from Dr. K. Nishida (Daichi Pharmaceuticals Co. Ltd., Tokyo) and was cultured in RPMI supplemented with L-glutamine and penicillin/streptomycin plus 10% fetal calf serum in an atmosphere of 95% air and 5% CO2 at 37 °C. Preincubation of the cells with anti-α4 antibody and RGDS peptides. Preincubation of the cells with anti-α4 antibody inhibited vortex-mediated cell adhesion to VCAM-1 by about 80%, but not with control IgG (Fig. 3A). Preincubation with RGDS, but not with REDS peptides, inhibited vortex-mediated cell adhesion to fibronectin (Fig. 3B). We also studied the change of β1 integrin expression on THP-1 cells induced by vortex-mediated mechanical stress, but we could not find any change of the expression by flow cytometry (data not shown). These data indicate that cell adhesion in our assay depends on the interaction between integrins and their ligands and that vortex-mediated mechanical stress increased the avidity or affinity of both α4β1 and α5β1 integrins in THP-1 cells.

**RESULTS**

**Vortex-mediated Mechanical Stress Increases Adhesion of THP-1 Cells to VCAM-1 and Fibronectin—**To determine the regulation of integrin avidity or affinity by mechanical stress mediated by vortex flow, we studied adhesion of THP-1 cells to purified adhesion molecules. Cell adhesion to soluble VCAM-1, soluble ICAM-1, and fibronectin was determined after cells were exposed to vortex flow for 5 s at 1,500 rpm to mimic vortices that may occur in the cardiovascular system (12, 13, 19). Vortex-mediated mechanical stress increased adhesion of THP-1 cells to VCAM-1 and fibronectin by approximately five-fold but not to ICAM-1 (Fig. 1).

Vortex-mediated cell adhesion to VCAM-1 and fibronectin increased in a speed-dependent manner (Fig. 2). To show that this cell adhesion is dependent on α4β1 and α5β1 integrins, we preincubated the cells with anti-α4 antibody and RGDS peptides. Preincubation of the cells with anti-α4 antibody inhibited vortex-mediated cell adhesion to VCAM-1 by about 80%, but not with control IgG (Fig. 3A). Preincubation with RGDS, but not with REDS peptides, inhibited vortex-mediated cell adhesion to fibronectin (Fig. 3B). We also studied the change of β1 integrin expression on THP-1 cells induced by vortex-mediated mechanical stress, but we could not find any change of the expression by flow cytometry (data not shown). These data indicate that cell adhesion in our assay depends on the interaction between integrins and their ligands and that vortex-mediated mechanical stress increased the avidity or affinity of both α4β1 and α5β1 integrins in THP-1 cells.

**Transient Integrin Activation after Vortex-mediated Mechanical Stress—**Next, we studied the time-dependent effect of vortex flow on cell adhesion to VCAM-1 and fibronectin. We found that vortex-mediated mechanical stress increased cell adhesion to both VCAM-1 and fibronectin quite rapidly, reaching a peak at 2–5 s of stimulation, indicating that such a brief vortex stimulation is enough to activate β1 integrin (Fig. 4). To examine reversibility of this integrin activation, cells were vortexed at 1,500 rpm for 5 s and left static for the indicated minutes. Cell adhesion to VCAM-1 or fibronectin was then determined. After the cells were left static for only 4 min, the cell adhesion induced by vortex flow was rapidly reduced to ~50% (Fig. 5), showing that this integrin activation induced by vortex-mediated mechanical stress is quite transient and reversible.
Integrin Activation Induced by Vortex-mediated Mechanical Stress Depends on IP$_3$-sensitive Ca$^{2+}$ Release from Intracellular Stores—Calcium signals are reported to be important for various cell responses such as integrin activation leading to cell adhesion (20). To determine whether Ca$^{2+}$ is involved in integrin activation induced by vortex-mediated mechanical stress, we next pretreated the cells with BAPTA-AM, an intracellular Ca$^{2+}$/H$^{+}$ chelator. Pretreatment of the cells with BAPTA-AM inhibited vortex-mediated cell adhesion to fibronectin (Fig. 6A) and VCAM-1 (data not shown), indicating that intracellular Ca$^{2+}$ is necessary for this integrin activation. To determine whether a stretch-activated Ca$^{2+}$/H$^{+}$ channel, a well known sensing system for mechanical stress (17), or Ca$^{2+}$ influx from the extracellular space is involved in integrin activation induced by vortex-mediated mechanical stress, we next pretreated the cells with GdCl$_3$	extsubscript{6}H$_2$O, a specific stretch-activated channel inhibitor, or NiCl$_2$	extsubscript{6}H$_2$O, a nonspecific Ca$^{2+}$ influx inhibitor. Pretreatment of cells with these inhibitors did not affect vortex-mediated cell adhesion to fibronectin (Fig. 6B) or VCAM-1 (data not shown), indicating that this integrin activation does not depend on stretch-activated channels or Ca$^{2+}$ influx from outside of the cells. These data indicate that Ca$^{2+}$ release from intracellular Ca$^{2+}$ stores such as endoplasmic reticulum may play a key role for this phenomenon.

Ca$^{2+}$ is released from the intracellular Ca$^{2+}$ stores via two known channels, one sensitive to inositol 1,4,5-trisphosphate (IP$_3$) and the other sensitive to ryanodine. Therefore, to determine the mechanism of Ca$^{2+}$ release from intracellular Ca$^{2+}$ stores, we pretreated the cells with thapsigargin, an inhibitor of Ca$^{2+}$-ATPase that inhibits IP$_3$-dependent Ca$^{2+}$ release from intracellular stores (21, 22). Because thapsigargin itself induces sustained elevation of intracellular calcium mediated by capacitative Ca$^{2+}$ influx (23, 24), we added NiCl$_2$ to block this Ca$^{2+}$ influx. Pretreatment of THP-1 cells with thapsigargin and NiCl$_2$ inhibited vortex-mediated mechanical stress-induced cell adhesion to fibronectin (Fig. 6B) and VCAM-1 (data...
Concentrations of U-73122 (\(\text{f}0\)) for 1 h in a non-atmosphere (95% air and 5% CO\(_2\) at 37 °C).

After incubation, cells were subjected to adhesion assays on fibronectin for 5 min with (open bar) or without (filled bar) vortexing at 1,500 rpm for 5 s. Data represent the mean ± S.D. of triplicate measurements from three independent experiments.

Mechanical stress depends on IP\(_3\)-sensitive Ca\(_{2+}\) release from intracellular stores. A, THP-1 cells were preincubated with 50 \(\mu \text{M}\) BAPTA-AM for 1 h in an atmosphere of 95% air and 5% CO\(_2\) at 37 °C.

Fig. 6. Integrin activation induced by vortex-mediated mechanical stress depends on IP\(_3\)-sensitive Ca\(_{2+}\) release from intracellular stores. A, THP-1 cells were preincubated with 50 \(\mu \text{M}\) BAPTA-AM for 1 h in an atmosphere of 95% air and 5% CO\(_2\) at 37 °C.

After incubation, cells were subjected to adhesion assays on fibronectin for 5 min with (open bar) or without (filled bar) vortexing at 1,500 rpm for 5 s. Data represent the mean ± S.D. of triplicate measurements from three independent experiments.

B, THP-1 cells were preincubated with 50 \(\mu \text{M}\) GdCl\(_3\)-ethanol for 1 h or 1 \(\mu \text{M}\) thapsigargin (THG) for 3 h in an atmosphere of 95% air and 5% CO\(_2\) at 37 °C. After incubation, cells were subjected to adhesion assays on fibronectin for 5 min with (open bar) or without (filled bar) vortexing at 1,500 rpm for 5 s. Data represent the mean ± S.D. of triplicate measurements from three independent experiments.

C and D, THP-1 cells were preincubated with the indicated concentrations of U-73122 (C) or ryanodine (D) for 1 h in an atmosphere of 95% air and 5% CO\(_2\) at 37 °C. After incubation, cells were subjected to adhesion assays on VCAM-1 or fibronectin for 5 min with (open bar) or without (filled bar) vortexing at 1,500 rpm for 5 s. Data represent the mean ± S.D. of triplicate measurements from three independent experiments.

Cell Adhesion to Fibronectin (O.D.)

Fig. 7. Calmodulin inhibitor inhibits vortex-induced adhesion to VCAM-1 and fibronectin in a dose-dependent manner. THP-1 cells were preincubated with W-7 (calmodulin inhibitor) at indicated concentrations for 2 h in an atmosphere of 95% air and 5% CO\(_2\) at 37 °C.

After incubation, cells were subjected to adhesion assays on VCAM-1 or fibronectin for 5 min with (open bar) or without (filled bar) vortexing at 1,500 rpm for 5 s. Data represent the mean ± S.D. of triplicate measurements from three independent experiments.

D, THP-1 cells were preincubated with W-7 at indicated concentrations for 2 h in an atmosphere of 95% air and 5% CO\(_2\) at 37 °C. After incubation, cells were subjected to adhesion assays on fibronectin for 5 min with (open bar) or without (filled bar) vortexing at 1,500 rpm for 5 s. Data represent the mean ± S.D. of triplicate measurements from three independent experiments.

Discussion

In this study we have examined the effect of vortex-mediated mechanical stress on integrin-dependent cell adhesion in human monocytic THP-1 cells and have clearly shown that a brief period of vortex-mediated mechanical stress activated β1 integrin, resulting in cell adhesion to VCAM-1 and fibronectin in a transient and reversible manner. We have also shown that IP\(_3\)-sensitive Ca\(_{2+}\) release from intracellular Ca\(_{2+}\) stores and calmodulin are involved in this integrin activation. This mechanism might explain why atherosclerosis is prone to progress in bifurcated or stenotic regions, and this may be a novel aspect of atherosclerosis and inflammation.

Most of the studies on mechanotransduction in the cardiovascular field have been done in endothelial cells and smooth muscle cells. The endothelial cells are normally subjected to mechanical stimuli from shear stress and from strain associated with stretch of the vessel wall. These stimuli can be detected by a mechanosensor that initiates a variety of signal transduction cascades (17, 27). For example, in response to the change in shear stress the endothelium can change the gene expression of various cytokines and adhesion molecules (15, 16, 28) that would be related to the promotion of atherosclerosis, thrombosis, and inflammation. Few studies, however, have been conducted to elucidate the changes in the adhesive property of leukocytes in the vortex flow, which might be also related to the induction of atherosclerosis. Fukuda et al. (11) have reported that human leukocytes respond to fluid shear stress by retracting pseudopods and down-regulate the integrin expression under the laminar flow condition, which would help leukocytes to run in the vessel wall. However, in the tortuous cardiovascular system, such as branching of the vessels and downstream of partially occluded vessels, leukocytes and plate-
lets can be subjected to differing shear forces under nonlaminar flow patterns (1, 12, 13). In this study, therefore, we exposed cells to vortex flow in order to mimic vortices that may occur in the cardiovascular system. In the study of platelet aggregation, a stirring bar has been used to expose platelets to vortex flow (19). Because it is important to expose whole cells to vortex flow instantaneously to mimic the in vivo situation, vortexing the cells in a vortex machine would be more reasonable to stimulate the cells in vitro. Establishing an in vivo model would be more important to show the relevance of this data to in vivo situations.

In previous studies, the endothelial intracellular Ca2+ concentration in response to mechanical stress is biphasic, consisting of an initial transient rise that depends on Ca2+ release from IP3-sensitive stores, followed by a sustained elevation mediated by Ca2+ influx (22, 29, 30). However, in this report we have shown that Ca2+ influx from the extracellular space is not necessary for integrin activation induced by vortex-mediated mechanical stress on THP-1 cells. Our data also clearly indicate that IP3-dependent Ca2+ release from intracellular Ca2+ stores plays a key role in this mechanism. Although the reason why only Ca2+ release from intracellular stores is required for vortex-mediated integrin activation remains unclear, it might be because of the shortness of vortex stimulation and integrin activation.

Calmodulin is a Ca2+ binding protein and is reported to be important for various cell responses, such as integrin activation leading to T cell adhesion (20) and aggregation (31). Our study clearly demonstrates that calmodulin also plays an essential role in regulating integrin activation induced by vortex-mediated mechanical stress as shown in various cell responses (32, 33). However, at present it is not clear how Ca2+ release from intracellular stores can be linked to the activation of calmodulin and integrin activation in THP-1 cells. Further studies, therefore, are required to clarify this mechanism.

In this study we have not been able to identify the sensing mechanism for vortex-induced mechanical stress in THP-1 cells. There is a possibility that a mechanosensor itself is not involved in this process. The forces applied at the cell surface might be transmitted to other locations via cytoskeleton. This kind of mechanotransduction is shown in the area of mechanical stretch (34). Therefore, an explanation of the sensing mechanism would be required to understand this process. Further understanding of how leukocyte adhesion functions in the tortuous cardiovascular system would enhance our knowledge of the nuances of the atherosclerotic and inflammatory process and should facilitate the development of drugs to regulate the process.

In summary, we have provided clear evidence that vortex-mediated mechanical stress on THP-1 cells quickly induces Ca2+- and calmodulin-dependent integrin activation, and IP3-dependent Ca2+ release from intracellular Ca2+ stores is involved in its mechanism. These findings might enlighten another aspect of increased atherosclerosis at stenotic or bifurcated regions.

Acknowledgment—We thank Hitomi Sagawa for excellent technical assistance.

REFERENCES

1. Turitto, V. T., and Hall, C. L. (1998) Thromb. Res. 92, suppl.) 25–31
2. Malek, A. M., Gibbons, G. H., Drau, V. J., and Izumo, S. (1993) J. Clin. Invest. 92, 2013–2021
3. Resnick, N., Yahav, H., Khachigian, L. M., Collins, T., Anderson, K. R., Dewey, F. C., and Gimbrone, M. A., Jr. (1997) Adv. Exp. Med. Biol. 430, 155–164
4. Mo, M., Eskin, S. G., and Schilling, W. P. (1991) Am. J. Physiol. 260, H1698–1707
5. Sriramaraoor, P., Languino, L. R., and Altieri, D. C. (1996) Blood 88, 3416–3423
6. Kroll, M. H., Hellums, J. D., McIntire, L. V., Schafer, A. I., and Moake, J. L. (1996) Blood 88, 1525–1541
7. Miyazaki, Y., Nishura, S., Miyake, T., Kagawa, H., Kitada, C., Taniguchi, H., Komiyama, Y., Fujimura, Y., Ikeda, Y., and Fukuhara, S. (1996) Blood 88, 3456–3464
8. Weiss, H. J., Hawiger, J., Ruggeri, Z. M., Turitto, V. T., Thiagarajan, P., and Hoffmann, T. (1989) J. Clin. Invest. 83, 288–297
9. Butcher, E. C. (1991) Cell 67, 1033–1036
10. Moazzam, F., DeLano, F. A., Zweifach, B. W., and Schmid-Schonbein, G. W. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 5338–5343
11. Fukuda, S., Yasu, T., Predescu, D. N., and Schmid-Schonbein, G. W. (2000) Circ. Res. 86, E13–18
12. Ku, D. N., Glagov, S., Moore, J. E., Jr., and Zarins, C. K. (1989) J. Vasc. Surg. 9, 309–316
13. Cao, J., and Rittgers, S. E. (1998) Ann. Biomed. Eng. 26, 190–199
14. Palmen, D. E., van de Vosse, F. N., Janssen, J. D., and van Dongen, M. E. (1994) J. Biomech. 27, 581–590
15. Mohan, S., Mohan, N., Valente, A. J., and Sprague, E. A. (1999) Am. J. Physiol. 276, C1100–1107
16. Nagel, T., Resnick, N., Atkinson, W. J., Dewey, C. F., Jr., and Gimbrone, M. A., Jr. (1994) J. Clin. Invest. 94, 885–891
17. Brekemeier, S., Eichler, I., Hopp, H., Kohler, R., and Hoyer, J. (2002) Cardiovasc. Res. 53, 209–218
18. Ashida, N., Arai, H., Yamasaki, M., and Kita, T. (2001) J. Biol. Chem. 276, 16555–16560
19. Soslowa, G., Schernher, A. J., Alcain, P. R., and Clark, R. (2000) Thromb. Res. 97, 15–27
20. van Kooij, Y., Weder, P., Heije, K., de Waal Malefijt, R., and Figdor, C. G. (1993) Cell Adhes. Comm. 1, 21–32
21. Yellowley, C. E., Jacobs, C. R., and Donahue, H. J. (1999) J. Cell. Physiol. 180, 402–408
22. Oke, M., Droogmans, G., and Nilius, B. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 2940–2944
23. Granfeld, D., Samuelsson, M., and Karlsson, A. (2002) J. Leukoc. Biol. 71, 611–617
24. Kurosawa-Matsumoto, M., Hirano, K., Ahmed, K., Kawakami, J., Kitada, C., Suzuki, S., and Kanaide, H. (2000) Br. J. Pharmacol. 131, 115–123
25. Sanderson, M. J., Charles, A. C., Botiano, S., and Dirksen, E. R. (1994) Mol. Cell. Endocrinol. 98, 173–187
26. Meissner, G. (1986) J. Biol. Chem. 261, 6300–6306
27. Lamsa, J. B., Hallam, T. J., and Rink, T. J. (1977) Nature 265, 811–813
28. Tebou, H., Ando, J., Korenaga, R., Takada, Y., and Kariya, A. (1995) Biochim. Biophys. Res. Commun. 206, 988–996
29. Geiger, R. V., Berk, B. C., Alexander, R. W., and Nerem, R. M. (1992) Am. J. Physiol. 262, C1411–1417
30. Helmingler, G., Berk, B. C., and Nerem, R. M. (1996) J. Vasc. Res. 33, 360–369
31. Andrews, R. K., Suzuki-Inoue, K., Shen, Y., Tulasne, D., Watson, S. P., and Berndt, M. C. (2002) Blood 99, 4219–4221
32. Exton, J. H. (1985) Am. J. Physiol. 248, E633–647
33. Johns, A., Linjten, P., Yamamoto, H., Hwang, K., and van Breemen, C. (1987) Am. J. Cardiol. 59, 13A–23A
34. Ingber, D. E. (1997) Annu. Rev. Physiol. 59, 575–599
Vortex-mediated Mechanical Stress Induces Integrin-dependent Cell Adhesion Mediated by Inositol 1,4,5-Trisphosphate-sensitive Ca^{2+} Release in THP-1 Cells
Noboru Ashida, Hajime Takechi, Toru Kita and Hidenori Arai

J. Biol. Chem. 2003, 278:9327-9331.
doi: 10.1074/jbc.M212316200 originally published online January 7, 2003

Access the most updated version of this article at doi: 10.1074/jbc.M212316200

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 34 references, 8 of which can be accessed free at
http://www.jbc.org/content/278/11/9327.full.html#ref-list-1