Effects of Estrogen Replacement Therapies on Mouse Platelet Function and Glycoprotein VI Levels

Xing-Hong Leng, Wei Zhang, Bernhard Nieswandt, Paul F. Bray

Clinical trials have shown estrogen replacement therapy (ERT) is associated with adverse arterial vascular events. Arterial thrombosis is initiated by platelet activation, but the in vivo effects of estrogens on platelet function are not well understood. We used a murine model of menopause to examine 3 major ERT regimes and test the hypothesis that ERT affects the intrinsic platelet response to agonists. The 3 ERT regimes studied were: (1) oral conjugated equine estrogen (CEE), (2) oral 17β estradiol (E2), and (3) subcutaneously implanted E2 (SQ E2). Paired ovariectomized littermates were treated with these regimes or placebo for 21 days. Two platelet agonists, thrombin and the GPVI-specific agonist collagen-related peptide (COL-RP), were used to evaluate platelet reactivity. Among the 3 regimes, (1) oral CEE enhanced platelet reactivity to COL-RP, (2) oral E2 had no effect on platelet reactivity to COL-RP and (3) SQ E2 increased platelet sensitivity to thrombin but lowered reactivity to COL-RP. Thus, the in vivo effects of estrogen on platelet function are agonist specific and dependent on hormone formulation and mode of delivery. The GPVI collagen receptor likely mediated some of these effects, because the ERT regimes induced changes in platelet surface GPVI expression corresponding to the observed platelet activation.

The results of randomized clinical trials of estrogen replacement therapy (ERT) in postmenopausal women have failed to demonstrate protection from cardiovascular events. ERT may adversely affect thrombosis, although the in vivo effects of estrogens on platelet function are not well understood. We used a murine model of menopause to examine 3 major ERT regimes and test the hypothesis that ERT affects the intrinsic platelet response to agonists. The 3 ERT regimes studied were: (1) oral conjugated equine estrogen (CEE), (2) oral 17β estradiol (E2), and (3) subcutaneously implanted E2 (SQ E2). Paired ovariectomized littermates were treated with these regimes or placebo for 21 days. Two platelet agonists, thrombin and the GPVI-specific agonist collagen-related peptide (COL-RP), were used to evaluate platelet reactivity. Among the 3 regimes, (1) oral CEE enhanced platelet reactivity to COL-RP, (2) oral E2 had no effect on platelet reactivity to COL-RP and (3) SQ E2 increased platelet sensitivity to thrombin but lowered reactivity to COL-RP. Thus, the in vivo effects of estrogen on platelet function are agonist specific and dependent on hormone formulation and mode of delivery. The GPVI collagen receptor likely mediated some of these effects, because the ERT regimes induced changes in platelet surface GPVI expression corresponding to the observed platelet activation.

Material and Methods

Effect of ERT on platelet function in vivo was tested using a murine model of menopause. ERT was administered to estrogen-deprived female mice for 21 days. Two platelet agonists, thrombin and the GPVI-specific agonist collagen-related peptide (COL-RP), were used to evaluate platelet reactivity. Among the 3 regimes, (1) oral CEE enhanced platelet reactivity to COL-RP, (2) oral E2 had no effect on platelet reactivity to COL-RP and (3) SQ E2 increased platelet sensitivity to thrombin but lowered reactivity to COL-RP. Thus, the in vivo effects of estrogen on platelet function are agonist specific and dependent on hormone formulation and mode of delivery. The GPVI collagen receptor likely mediated some of these effects, because the ERT regimes induced changes in platelet surface GPVI expression corresponding to the observed platelet activation.

Acknowledgments

This work was supported by grant RO1-CA126611 from the National Cancer Institute. The authors thank Debra Williams and Laura Wiles for their expert technical assistance.

References

1. From Medicine/Thrombosis Research (X.-H.L., W.Z., P.F.B.), Baylor College of Medicine, Houston, Tex; and Vascular Biology (B.N.), Rudolf Virchow Center for Experimental Biomedicine, University of Würzburg, Germany.

2. Correspondence to Paul F. Bray, MD, Thrombosis Research Section, Baylor College of Medicine, One Baylor Plaza, BCM 286, N1319, Houston, TX 77030. E-mail pbray@bcm.tmc.edu (Circ Res. 2005;97:415-417.)

© 2005 American Heart Association, Inc.

Circulation Research is available at http://circres.ahajournals.org
DOI: 10.1161/01.RES.0000181025.43762.cf

415
Results and Discussion

These littermate- and placebo-controlled studies were designed to test the in vivo effects of different clinically relevant estrogen preparations and routes of estrogen administration on intrinsic platelet function. Orally administered CEE and E2 restored the uteri to a normal physiologic size (online Table, available at http://circres.ahajournals.org), appearance and weight. CEE increased COL-RP–induced platelet fibrinogen binding (Figure 1A) and aggregation (Figure 1B), but oral E2 had no such effects (Figure 1). These results suggest that a non-E2 component of CEE may be responsible for the enhanced platelet reactivity to COL-RP. SQ E2 restored the uterus size to high-normal size (online Table) in the presence of physiologically normal levels of E2 (Methods), but caused a reduction in platelet reactivity to COL-RP. Tail bleeding times were only measured in the SQ study, where E2 treated mice showed a 344 ± 98% mean increase in bleeding time compared with placebo (P = 0.006), consistent with the reduced response to CRP after SQ E2. These differential effects of ERT on platelet function attributable to the route of estrogen administration are strikingly similar to the ERT effects on plasma triglycerides levels, which are increased by oral E2 and decreased by transdermal E2.9

In an effort to address the possible mechanisms by which estrogens might affect platelet function, we measured levels of adhesive membrane GPs. No difference between placebo and any estrogen therapy was observed for GPIbα and GPIIb-IIIa (integrin αIIbβ3). However, the 3 ERT regimens altered platelet surface GPVI levels in a pattern similar to COL-RP–induced platelet activation (Figure 2A). A strong correlation between GPVI expression and COL-RP–induced platelet fibrinogen binding (n = 11 pairs). MFI indicates mean fluorescence intensity; probability values, treatment vs placebo.

GPVI-IIIa (integrin αmβ3). However, the 3 ERT regimens altered platelet surface GPVI levels in a pattern similar to COL-RP–induced platelet activation (Figure 2A). A strong correlation between COL-RP–induced platelet activation and surface GPVI level was observed in these studies (Figure 2B), suggesting the changes in COL-RP–induced platelet reactivity caused by ERT are mediated in part via GPVI or its downstream signaling pathways.

This is the first report of ERT regulating GPVI expression and the lack of an ERT effect on GP Ibα or GPIIb-IIIa expression (Figure 2A) suggests hormone-specific effects on GPVI expression. The pivotal role of the platelet GPVI collagen receptor in platelet activation and thrombus formation has been demonstrated using selective agonists and GPVI-deficient platelets.8 Modest reductions in GPVI density (∼12% less than normal platelet levels) can have a major effect on cell adhesion under high shear conditions.10 Estrogens could act directly on megakaryocytes to regulate GPVI expression (eg, GPVI transcription or altering the expression of a metalloproteinase that induces platelet GPVI shedding). Because our assays assessed the in vivo effects of estrogens,
we cannot exclude an indirect effect whereby estrogen could induce nonmegakaryocyte cells to secrete a factor that could regulate platelet GPVI expression.

Additional platelet activation in vivo likely occurs after platelet adhesion, and we also examined the effect of thrombin on platelets from the ERT-treated mice. Neither oral regimen had a significant influence on fibrinogen binding (Figure 3), whereas SQ E2 increased platelet sensitivity to thrombin.11 Interestingly, the responses to COL-RP and thrombin were not concordant, indicating estrogens influence platelet function in an agonist specific fashion.

It is important to note that any mechanism whereby estrogen affects platelet function could be influenced by at least 3 variables: non-E2 estrogens in CEE, higher plasma levels of E2 after SQ E2, or the differences attributable to peaks and troughs of oral estrogens compared with the steady levels of SQ estrogens. In addition, because oral estrogen results in high portal vein estrogen levels, at least some of the estrogen effects could be mediated by first-pass metabolism in the liver and altered hepatic gene expression.

In summary, we have shown that in vivo estrogens affect platelet reactivity in an agonist-specific fashion and depend on the hormone formulation and mode of delivery. Altered GPVI expression likely mediated some of these effects. Cautiously extrapolating to postmenopausal women using ERT, these estrogen-induced platelet functional changes could contribute to cardiovascular complications. More human studies are needed to assess specific aspects of vascular complications of hormone therapy; specifically, does oral E2 lack the reported risks of CEE? And is there risks related to daily fluctuations of estrogen levels?

Acknowledgments
This study was supported in part by American Heart Association Scientist Development Grant 0335057N (Leng), Baylor College of Medicine, and the Fondren Foundation.

References
7. Leng XH, Hong SY, Larrucea S, Zhang W, Li TT, Lopez JA, Bray PF. Platelets of female mice are intrinsically more sensitive to agonists than are platelets of males. Arterioscler Thromb Vasc Biol. 2004;24:376–381.
8. Leng XH, Hong SY, Larrucea S, Zhang W, Li TT, Lopez JA, Bray PF. Platelets of female mice are intrinsically more sensitive to agonists than are platelets of males. Arterioscler Thromb Vasc Biol. 2004;24:376–381.

KEY WORDS: estrogen • glycoprotein VI • hormone replacement therapy • platelets

Figure 3. Effects of estrogen regimens on thrombin-induced platelet fibrinogen binding. Thrombin (0.005U/mL) induced fibrinogen (FGN) binding to washed platelets is shown. Percent change from placebo is shown for 4 to 13 estrogen/placebo pairs. Probability values indicates treatment vs placebo.
Effects of Estrogen Replacement Therapies on Mouse Platelet Function and Glycoprotein VI Levels
Xing-Hong Leng, Wei Zhang, Bernhard Nieswandt and Paul F. Bray

_Circ Res._ 2005;97:415-417; originally published online August 11, 2005;
doi: 10.1161/01.RES.0000181025.43762.cf

_Circulation Research_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2005 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the
World Wide Web at:
http://circres.ahajournals.org/content/97/5/415

Data Supplement (unedited) at:
http://circres.ahajournals.org/content/suppl/2005/08/11/01.RES.0000181025.43762.cf.DC1

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in _Circulation Research_ can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the _Permissions and Rights Question and Answer_ document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to _Circulation Research_ is online at:
http://circres.ahajournals.org/subscriptions/
Online Table. Characteristics of ovariectomized mice receiving estrogen therapies.

<table>
<thead>
<tr>
<th>Study →</th>
<th>Oral CEE vs. placebo</th>
<th>Oral E2 vs. placebo</th>
<th>SQ E2 vs. placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td># of pairs</td>
<td>% change*</td>
<td>P</td>
</tr>
<tr>
<td>Uterus weight†</td>
<td>13</td>
<td>+86.7 ± 16.8</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Platelet count</td>
<td>13</td>
<td>+10.3 ± 6.0</td>
<td>.114</td>
</tr>
<tr>
<td>Platelet size</td>
<td>13</td>
<td>+1.7 ± 1.4</td>
<td>.237</td>
</tr>
</tbody>
</table>

*Percent change from the placebo value.

† Uteri after oral ERT were in normal size range of the diestrum cycle; uteri after SQ E2 was in the normal size range of late metoestrum cycle.