Factor VIIa/tissue factor-dependent gene regulation and pro-coagulant activity: Effect of factor VIIa concentration

Lars C. Petersen, Tatjana Albrektsen, Gertrud M. Hjortø, Marianne Kjelke, Søren E. Bjørn, Brit B. Sørensen
Health Care Discovery, Novo Nordisk A/S, Maaloev, Denmark

Dear Sir,

In addition to its well known function as an initiator of coagulation, tissue factor (TF) has been implicated in tissue repair processes and in a variety of physiological and pathophysiological states where repair mechanisms are activated. TF may thus play a role in wound healing, angiogenesis, sepsis, inflammation, atherosclerosis, and tumour growth and metastasis (1–4). The exact function of TF in these inflammatory and pathophysiological processes is poorly understood. TF expression can be rapidly induced in several cell types as part of a normal tissue injury response, whereas aberrant expression of TF in cells that do not normally express TF seems to play a role e.g. in atherosclerotic plaque formation (5) and in tumor growth and metastasis (6–8). The pathogenesis of diseased states with increased TF expression and exposure to factor VII (FVII) and activated FVII (FVIIa) is associated with local pro-coagulant activity. However, accumulating data also implicates non-coagulant reactions mediated through TF/FVIIa-induced intracellular signalling (9–14) and gene transcription (15–19). Thus, complexes of TF/FVIIa may promote coagulation-dependent pathological effects leading to thrombosis, and in addition TF/FVIIa may promote coagulation-independent, cellular changes pertinent to aggravated inflammation or tumour progression.

The FVIIa concentration dependency of the pro-coagulant activity on TF-expressing cells has been extensively studied (20–23) and has been reported to be saturated at sub-nanomolar concentrations. In contrast, the intracellular response to FVIIa stimulation apparently requires higher FVIIa concentrations for saturation (24). Recent studies have indicated that TF-dependent pro-coagulant activity and TF-dependent cell signalling is mediated by two pools of cell surface TF that may reflect differentiation in distinct TF-dependent activities and/or TF “encryption/decryption” (25). It is, however, unclear whether the FVIIa concentration dependencies of the two TF-specific activities are in fact distinctly different when measured under controlled conditions on the same cell line. For clarification the FVIIa saturation kinetics of both activities on MDA-MB-231 breast carcinoma cells was studied. Based on this it was investigated whether rFVIIa, when added as a therapeutic haemostatic agent, is likely to enhance endogenous TF-dependent cellular transcriptional activity.

MDA-MB-231 cells express high levels of TF and protease activatable receptor type 1 and 2 (PAR1 and PAR2); and treatment of these cells with FVIIa induces a marked transcriptional regulation of IL-8, Gro-α and GM-CSF (26). Real-time qPCR measurements of these genes were applied to quantify the response at various concentrations of FVIIa. Figures 1A-C show that stimulation by FVIIa induced up-regulation of all three genes in a dose-dependent manner. FVIIa induction of gene transcription was saturable with EC50 values of 8.1 nM (n=1); 5.1 ± 1.3 nM (n=3); and 4.3 ± 1.8 nM (n=2) for IL-8, Gro-α and GM-CSF, respectively. Similar EC50 values of 7.6 ± 3.2 nM (n=3); 5.1 ± 0.5 nM (n=3); and 5.8 ± 1.7 nM (n=2) were obtained when the cells were treated with FVIIa in the presence of 100 nM FX. Only at low FVIIa concentration did the presence of FX significantly enhance FVIIa-induced gene transcription, suggesting that complexes of TF/FVIIa/FXa and TF/FVIIa are equipotent in inducing signal transduction in MDA-MB-231 cells under FVIIa-saturating conditions. The present EC50 value for TF/FVIIa-dependent gene regulation corroborated well with previous estimates based on measurements of FVIIa-induced inositol-3-phosphate hydrolysis (24), MAPK phosphorylation (14, 27) and IL8 gene regulation measured at mRNA and protein levels (28).

In order to directly compare FX-activation with FVIIa/TX signalling on the same cell line under closely similar conditions the EC50 for the FVIIa-catalyzed FX activation on TF-expressing MDA-MB-231 cells was determined (Table 1). TF is expressed at high levels by MDA-MB-231 cells (29), whereas human vascular smooth muscle cells (VSMCs) express TF at a reduced level, and LPS-stimulated monocytes express TF at a further reduced level. We therefore went on to examine whether the FVIIa concentration-dependency of FX activation was affected by cell type and TF expression level. The results (Table 1) confirm previous observations (20, 23, 30) showing that TF-dependent FX activation is saturated at very low concentrations of FVIIa. Ionomophores promote cellular blebbing, TF exposure and increased FVIIa-mediated FX activation (31, 32). Addition of 10 μM ionomycin to MDA-MB-231 cells induced a 10-fold enhancement of FX activation (equally distributed between supernatant and adherent cells) without a significant change in the EC50 for FVIIa saturation (results not shown). This suggests that “decryption” of TF does not increase the EC50 to the level observed for signalling. The EC50 values obtained were in the picomolar range (= 0.02 nM) for all three cell types, and two to three orders of magnitudes lower than the EC50 of about 5 nM which characterized FVIIa/TX-induced gene regulation by MDA-MB-231 cells. The latter EC50 value is close to the Ki of 2 ± 1 nM for binding

Correspondence to:
Lars C. Petersen, PhD, DSc
Haemostasis Biology, Health Care Discovery
Novo Nordisk A/S, Maaloev, Denmark
Tel.: +45 44434345, Fax: +45 44434347
E-mail: LCP@novonordisk.com

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Letters to the Editor

Recombinant FVIIa (rFVIIa) at supra-physiological levels is approved for treatment of bleeding episodes in inhibitor-complicated haemophilia patients and has the potential to reduce haemorrhage in non-haemophilia patients with critical bleeds (33, 34). The haemostatic action of high level rFVIIa involves enhancement of the propagation phase of coagulation by a TF-independent process driven by FVIIa-mediated activation of FX on locally activated platelets (35, 36) although this has been a matter of controversy (37, 38). However, since high level FVIIa may be administered in certain clinical settings with dispersed TF expression (39, 40) it is important to consider putative TF-dependent pathogenic implications in light of the present data on FVIIa saturation of pro-coagulant and non-coagulant activities. The endogenous level of FVII zymogen is 10 nM whereas approximately 1% (0.1 nM) circulates as the activated form, FVIIa (41). The low EC\textsubscript{50} for the pro-coagulant activity ensures near saturation of TF-mediated FX activation at 0.1 nM FVIIa. The presence of plasma FX (136 nM) and the existence of FX-mediated feed-back activation of FVII ensures efficient accumulation of the TF-FVIIa complex (42, 43) with a presumed saturation of TF corresponding to the FVII level. We confirmed with MDA-MB-231 cells that the FX activation rate was identical, whether initiated with 10 nM FVIIa or a mixture of 0.1 nM FVIIa and 10 nM FVII (results not shown). Thus, our data on FVIIa-mediated FX activation using various TF-expressing cells suggest that the TF-dependent procoagulant activity of FVIIa reaches its optimum at concentrations far below plasma FVIIa/FVII levels (>99% saturation) and is not significantly increased by therapeutic use of rFVIIa in the management of critical bleeding. Near saturation (61–88%) of TF-dependent FVIIa stimulation was also attained at FVIIa/FVII plasma levels for the cellular response of MDA-MB-231 cells, suggesting that supra-physiological levels are unlikely to enhance gene transduction much beyond that induced by the endogenous FVII/FVIIa level. Although in-vitro studies of course may not fully account for the complex situation in vivo, our data suggest that upon exposure to the normal plasma milieu, TF is likely to be engaged in near maximal TF/FVIIa activity with respect to both pro-coagulant and non-coagulant processes.

Table 1: EC\textsubscript{50} values for FVIIa-mediated FX activation on TF-expressing cells. Monocytes were isolated from citrate-stabilized peripheral blood taken from healthy donors and were stimulated and prepared as previously described (30). The VSMC-line was a generous gift from Dr. H. Versteeg, The University of Amsterdam.

<table>
<thead>
<tr>
<th>Cell line</th>
<th>EC\textsubscript{50} (nM)</th>
<th>Max FXa generation (nM/min)</th>
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<tbody>
<tr>
<td>MDA-MB-231</td>
<td>0.035 ± 0.008</td>
<td>2.0 ± 0.8</td>
</tr>
<tr>
<td>VSMC</td>
<td>0.022 ± 0.006</td>
<td>0.14 ± 0.04</td>
</tr>
<tr>
<td>LPS stimulated monocytes</td>
<td>0.006 ± 0.002</td>
<td>0.04 ± 0.01</td>
</tr>
</tbody>
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*Mean ± SD, n = 3–4. Calculation of EC\textsubscript{50} values is based on data from no series where cells within the same day were stimulated with various concentrations of FVIIa. The same approach was applied for calculation of EC\textsubscript{50} values for FVIIa-induced gene regulation listed in the text.

of \textsuperscript{125}I-FVIIa to TF on MDA-MB-231 cells (unpublished results) and to K\textsubscript{D} values reported for binding of FVIIa to TF on other cell types (21). This may suggest that the majority of TF capable of binding FVIIa, possibly including “encrypted” TF (21), is active in relation to FVIIa/TF-mediated signalling. It does not, on the other hand, exclude signal transduction by FVIIa bound to pro-coagulant “decrypted” TF on MDA-MB-231 cells capable of FX activation or signalling by the ternary T/FVIIa/FXa complex.

Figure 1: Transcriptional regulation of IL-8 (A), CXCL-1 (B), and GM-CSF (C) in MDA-MB-231 cells induced by stimulation with FVIIa in the presence and absence of FX. Total RNA from MDA-MB-231 cells treated with increasing concentrations of FVIIa in the absence (○) or presence (●) of 100 nM FX for 3 hrs was isolated and subjected to real time quantitative qPCR analysis (26). Regulation of mRNA levels is presented as ratios relative to media controls and represents the mean ± SEM (n = 3) from three independent experiments. *denotes a statistically significant increased mRNA level (p<0.05) induced by FVIIa in the presence of 100 nM FX as compared with the level induced by the same FVIIa concentration in the absence of FX. Controls indicating lack of a significant effect by stimulating MDA-MB-231 cells with 100 nM FX (△) or 100 nM FXa (○) in the absence of FVIIa are shown for comparison (superimposed in B and C).
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