Tissue-Type Plasminogen Activator: A Role for O-linked Fucose

Editorial

Tissue-type plasminogen activator (t-PA) is used clinically for the dissolution of thrombi in coronary arteries during the acute stages of myocardial infarction (1). A proposed merit of t-PA is that it catalyzes the conversion of plasminogen to plasmin at the site of the thrombus and should, as a result, be more selective and have fewer complications. The short half-life of t-PA in plasma, ~ 5 min in humans, however, limits its successful clinical application. The short circulatory half-life is characteristic of both recombinant and native forms of t-PA and is a result of rapid clearance by the liver. Establishing the mechanism for rapid clearance by the liver is of considerable interest from many perspectives.

Three distinct mechanisms that may contribute to t-PA clearance have been described: (*a*) binding to the mannose-specific receptor of liver endothelial cells (2), (*b*) plasminogen activator inhibitor type 1 (PAI-1)-dependent binding to liver parenchymal cells (3); and (*c*) binding to the low density lipoprotein receptor–related protein/α_2-macroglobulin receptor (LRP receptor) on liver parenchymal cells (4). In this issue of *The Journal* Hajjar and Reynolds (5) report an additional potential mechanism based on a highly novel saccharide thus far found within the EGF domains of the coagulation/fibrinolytic proteins t-PA, urokinase, factor VII, factor XII, and factor IX. Each of these proteins has a single fucose in α-linkage to either a Ser or Thr residue within the sequence Cys-X-X-Gly-Gly-Thr/Ser-Cys (6). Suspended HepG2 cells, a well-differentiated human hepatoma cell line, bind t-PA with a K_d of 39 nM and a B_{max} of 493,000 sites per cell. These cells rapidly internalize and degrade bound t-PA at 37°C. Based on inhibition studies and digestion with α-fucosidase, the authors conclude that binding requires the presence of the O-linked fucose. Binding is Ca^{2+} dependent, suggesting the binding activity may be related to the C-type lectins, which require Ca^{2+} for binding (7).

Many functions have been proposed for glycosylation (8). The presence of unique structures on individual glycoproteins suggests that either these saccharides will be recognized by highly specific receptors and/or that they selectively modulate a functionally important property of the underlying protein. As an increasing number of mammalian carbohydrate binding proteins have been described, the role of oligosaccharides in directing specific forms of recognition has been enhanced. Frequently, however, we are left with oligosaccharides in search of receptors and receptors in search of oligosaccharides; the challenge for glycobiologists is to establish the biologic targets for these receptors and oligosaccharides. The possibility that t-PA clearance is mediated by fucose-specific recognition is an exciting one for glycobiologists who are frequently asked, "So what do they do?"

What remains is to establish if this represents a significant mechanism for the clearance of t-PA in vivo. This could be done most simply by determining the clearance rate for t-PA that has had its fucose removed by digestion with α-fucosidase, as was done for the in vitro studies with HepG2 cells. Both t-PA and t-PA/PAI-1 complexes are bound by the LRP receptor, and a 39-kD protein that inhibits binding to the LRP receptor in vitro prolongs the in vivo circulatory life of t-PA in rats (4). This strongly implicates the LRP receptor in t-PA clearance in vivo and suggests that the contribution of PAI-1 to clearance of t-PA may be relatively minor. Although the authors have shown that PAI-1 does not contribute to recognition on the basis of fucose, it is possible that binding to the LRP receptor is dependent on the O-linked fucose. If it is not, two entirely independent mechanisms for t-PA clearance must exist.

There are now several examples of glycoproteins whose circulatory half-life is controlled by oligosaccharide-specific receptors. Rapid removal from the circulation may in many instances be essential to prevent unwanted side effects away from the actual site of action of glycoproteins such as coagulation/fibrinolytic factors. For more complex structures, variations in valency, structure, and location of oligosaccharides have the potential to modulate interaction with receptors. For a simple structure such as the one found on t-PA such variations seem unlikely, and one might expect that virtually all glycoproteins bearing these structures will be recognized to a similar degree. Thus, if the in vivo clearance of t-PA is mediated by O-linked fucose its behavior should be a model for virtually all glycoproteins bearing these structures. Stimulated by these observations, other glycobiologists with unique oligosaccharide structures should feel encouraged to search for specific receptors so that we can at last respond to the question of function by pointing out that oligosaccharides are essential for intercellular communication and targeting in multicellular organisms.

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References

1. Lijnen, H. R., and D. Collen. 1991. Strategies for the improvement of thrombolytic agents. *Thromb. Haemostasis.* 66:88–110.

2. Otter, M., M. M. Barrett-Bergshoeff, and D. C. Rijken. 1991. Binding of tissue-type plasminogen activator by the mannose receptor. *J. Biol. Chem.* 266:13931–13935.

3. Morton, P. A., D. A. Owensby, T.-C. Wun, J. J. Billadella, and A. L. Schwartz. 1990. Identification of determinants involved in binding of tissue-type plasminogen activator-plasminogen activator inhibitor type 1 complexes to HepG2 cells.. *J. Biol. Chem.* 265:14093–14099.

4. Warshawsky, I., G. Bu, and A. L. Schwartz. 1993. 39-kD protein inhibits tissue-type plasminogen activator clearance in vivo. *J. Clin. Invest.* 92:937–944.

5. Hajjar, K. A., and C. M. Reynolds. 1993. α-Fucose-mediated binding and degradation of tissue-type plasminogen activator by HepG2 cells. *J. Clin. Invest.* 93:703–710.

6. Harris, R. J., C. K. Leonard, A. W. Guzzetta, and M. W. Spellman. 1991. Tissue plasminogen activator has an O-linked fucose attached to threonine-61 in the epidermal growth factor domain. *Biochemistry.* 30:2311–2314.

7. Drickamer, K . 1991. Clearing up glycoprotein hormones. *Cell.* 67:1029–1032.

8. Varki, A . 1993. Biological roles of oligosaccharides: All of the theories are correct. *Glycobiology.* 3:97–130.

J. Clin. Invest.

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