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GLYCOPINION

Editor: RAYMOND A. DWEK

Readers of the article by Dr Kenneth Seamon (Federal Drug Association) in last month's Glycopinion raised a number of points:

- Are the biochemical processes involved in glycosylation so heterogeneous and haphazard that there is little we can do to control them?
- Is the natural spectrum of glycosylated variants so precise and unique that it cannot be reproduced *in vitro*?
- Does it matter if the glycosylation of a therapeutic differs from that of the native glycoconjugate?
- What are the significant features of glycosylation that should be defined by analysis?
- How can the improved efficiency of a drug be measured?

As Dr T. Rademacher (Oxford Glycobiology Institute) pointed out: "It recently took a study of over 25000 patients to compare the effects of aspirin, t-PA and streptokinase for their ability to reduce mortality post-coronary thrombosis. Does the FDA expect a new version of t-PA to be tested side by side with the propriety band before giving approval? The superior efficacy test is simply not workable and will prevent new and better drugs reaching the market."

The following article addresses some of these questions and discusses the impact which automated oligosaccharide analysis technology will make on our understanding of glycosylation.

Automation of glycosylation analysis: a way forward for recombinant therapeutics

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Dr Seamon is to be congratulated on a careful and cogent presentation of many of the key issues concerning glycosylation of therapeutic recombinant glycoproteins. Any regulatory agency or company involved in developing such products is bound by the dual considerations of minimizing interference and delay in bringing products of therapeutic value to the clinic while seeking to maximize the safety of such products during sustained and repeated production and administration. In what is becoming an increasingly controversial issue, it is easy to lose sight of some of the central scientific issues concerning glycosylation of therapeutic proteins. Dr Seamon does well to return the discussion to the main track.

In developing and administering recombinant glycoproteins, numerous structural and biological aspects need to be considered, e.g. glycosylation. In deciding whether glycosylation analysis of recombinant glycoproteins is necessary, and in what depth, it is worth bearing in mind four points.

First, glycosylation *is* important to the *in vivo* (although not always *in vitro*) properties of a therapeutic protein. Numerous studies now indicate that both the number of attached glycan chains as well as their precise structures can influence the specific activity, pharmacokinetics, distribution and immunogenicity of a polypeptide.

Second, a recombinant glycoprotein does *not* need to be structurally identical to the native form. Indeed, the precise glycosylation of the native glycoprotein is not a particularly meaningful concept in developing therapeutic products since this glycosylation is cell type-specific, tissue-specific, dependent on physiological and pathological status, and even on race and the individual source. What then should the 'reference' glycosylation pattern of the native form be considered to be? It is more important at present that the glycosylation of a recombinant glycoprotein be shown to be consistent with protein efficacy and 'compatible' with the intended recipient.

Third, oligosaccharides are fundamentally different from protein and nucleic acids with respect to structure, biosynthesis and occurrence. This increased structural complexity together with a lack of correspondingly precise analytical tools makes an analysis of glycosylation to the

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same level as for proteins and nucleic acids beyond the capacity of all but a few laboratories. For recombinant glycoproteins, it therefore becomes necessary to decide the level of glycosylation analysis that is both practical and sufficient for each stage of product development and production, and to bear clearly in mind why this analysis is being performed.

Fourth, consistency of glycosylation is essential during repeated production of a recombinant glycoprotein. Just as one would be reluctant to allow variations in amino acid sequence of a polypeptide (even though one usually does not know the precise function of each individual amino acid), so with glycans. Oligosaccharides do influence biological function often in an unpredictable way and variation in glycosylation, from a glycosylation pattern known to be compatible with *in vivo* administration, should be avoided.

By considering glycosylation from the earliest stages of development of a therapeutic recombinant glycoprotein, many of the problems encountered late in the product development cycle can be avoided. The following discussion seeks briefly to expand this point. At a relatively early stage in developing a therapeutic recombinant protein, a decision is made as to whether the recombinant product needs to be glycosylated for it to show biological activity. In this stage it is clearly necessary to produce glycosylation variants (either by isolation from different sources or enzymatic modifications) of the glycoprotein of interest and to correlate biological activity (almost invariably *in vitro*) to the presence, absence or precise structure of glycan chains. Glycosylation analysis will therefore involve monosaccharide composition analysis (to determine the glycan content of each variant), glycan 'mapping' (whether at individual glycosylation sites or not) to assess glycan heterogeneity and some form of structural characterization (although not necessarily full sequence analysis) to determine for example sialic acid content, relative incidence of *N*- and *O*-glycans, and relative content of each class of *N*-glycans. Correlation of glycosylation to biological activity of even a relatively few variants can provide enormous information on the acceptable forms of glycosylation of that product. For example, de-*N*- and/or de-*O*-glycosylation can establish the need for either of these types of glycan chains. If *N*-glycans are required for activity, is it complex-type chains or high mannose ones that are needed? If complex-type chains are needed, should they be sialylated? In this way glycosylation patterns that are necessary for activity can be defined and considered in choosing acceptable cell expression systems. It must always be borne in mind at this stage, that if glycosylation of a protein that is glycosylated in its native form is not necessary for the desired activity *in vitro*, it may be necessary for good pharmacokinetics *in vivo* and to prevent exposure to the immune system of peptide determinants that are normally 'masked' by carbohydrate.

Once an expression system is chosen, it then becomes useful to establish that it does not express carbohydrate determinants that are 'incompatible' with the recipient, i.e. ones which induce an immune reaction or cross-react with naturally occurring antibodies (and possibly also endogenous lectins). A 'rogues gallery' of such determinants, including various blood group structures, can now be drawn up and is constantly being expanded. It may increase confidence in the ultimate efficacy of a product to screen early on for such determinants and, if any are found, to make a reasoned decision as to whether to eradicate them from the final product or to retain them. Analysis for such determinants is preferably performed by direct structural analysis of glycans or, failing that, by the use of lectins or antibodies.

Development of a production process for a recombinant glycoprotein can only really be considered validated when it is shown that the process reproducibly conserves all the structural characteristics of the reference compound. During this stage the preferred methods of glycosylation analysis involve both a determination of monosaccharide composition (to measure the overall glycan content) and glycan 'mapping' (to measure any variations in glycosylation pattern). Monosaccharide composition analysis, by virtue of the 'averaging' that occurs prior to measurement, is not a particularly useful method for comparative analysis of glycosylation patterns. Often glycan 'mapping' is not quantitative. Similar considerations apply during repeated manufacturing production of serial batches.

In summary, accepting that glycosylation of a therapeutic protein may be necessary for its efficacy allows one to incorporate glycosylation analysis into the normal product development cycle and to avoid 'glycosylation-related' problems at a late stage. The techniques for performing such analysis are available (although not yet in a fully automated form) and much of the necessary analysis can be performed relatively simply without very sophisticated instrumentation or great technical expertise. The knowledge about the product so gained will almost invariably prove more useful in the long run than ignorance.

Biochemical analysis, particularly in the industrial sector, is increasingly dependent on automated instrumentation. A barrier to performing certain analyses on a routine basis is not always the lack of suitable methods, but rather the lack of their automation. It is possibly useful, therefore, to invest effort in developing dedicated analytical instruments for glycosylation analysis – this is analogous to the advances in protein analysis. As methods for amino acid composition analysis, peptide mapping and eventually sequencing became routine and were automated, so more sophisticated analyses could be incorporated into the process for recombinant protein production. It is likely that much of the controversy surrounding glycosylation of recombinant therapeutics will disappear as automated technologies for reliable, reproducible and accurate analysis of protein glycosylation become available.

In conclusion, glycosylation analysis and correlation of

carbohydrate structure to glycoprotein function are playing an increasing part in the development and production of recombinant therapeutics. The nature of the analysis is and will continue to be largely dependent on the technology that is widely available. Dr Seamon and his colleagues at the FDA are providing a useful lead in increasing the awareness of the therapeutic significance of protein glycosylation, in establishing a framework for incorporating glycosylation analysis into the entire development process and in urging efforts to increase the sensitivity and reliability of analytical methods.

Letters or comments relating to this article would be received with interest by Pauline Rudd, Assistant to the Special Advisory Editor, R. A. Dwek.