The number of people with diabetes is expected to rise to approximately 300 million by the year 2030; of this number some 90% will have the type 2, non-insulin dependent form of the disease.1 The major goal in the treatment of type 2 diabetes is to achieve and maintain glycaemic control, since episodes of hyperglycaemia are associated with the risk of microvascular and macrovascular complications.2 Treatment for type 2 diabetes is typically incremental, starting with the modification of diet and introduction of exercise followed by the introduction of an orally active diabetic medication.3 Despite the wide range of available oral medications, many patients fail to achieve appropriate glycaemic control and, in the absence of formulations and devices on the market that can deliver insulin via non-injectable routes, will ultimately require the introduction of injectable insulin.

A particular focus area research for the treatment of type 2 diabetes is the development of alternative and supportive therapies to oral diabetic agents that reduce the need for the introduction of insulin. In particular, analogues of the natural GLP-1 peptide have become important class of molecules in this area.3 GLP-1 is a 30 amino acid peptide that belongs to the incretin family. It is secreted in direct response to the ingestion of food and acts to both stimulate insulin secretion and decrease glucagon production providing an effective mechanism for glycaemic control. In addition, the peptide also acts to increase satiety and decrease gastric emptying which in turn lead to moderate weight loss. Taken together these factors have led to

“DESPITE THE WIDE RANGE OF AVAILABLE ORAL MEDICATIONS, MANY PATIENTS FAIL TO ACHIEVE APPROPRIATE GLYCAEMIC CONTROL AND, IN THE ABSENCE OF FORMULATIONS AND DEVICES ON THE MARKET THAT CAN DELIVER INSULIN VIA NON-INJECTABLE ROUTES, WILL ULTIMATELY REQUIRE THE INTRODUCTION OF INJECTABLE INSULIN”
considerable interest in using this molecule as an anti-diabetic therapeutic. A half-life of native GLP-1 is around two minutes; a significant issue when considering this molecule as a therapeutic. The short half-life of GLP-1 is caused by specific clearance by a peptidase enzyme (DPP-4) and by renal clearance due to its relatively small size. The therapeutics that have reached the market or are currently achieving success in the clinic are based on GLP-1 analogues or mimics that have been designed to overcome the issues observed for the native GLP-1 molecule. These can be divided into short and long acting and a summary of these is detailed in Figure 1.

A common feature in the advances in dosing regimen made by Liraglutide (short-acting), Albiglutide and CJC-1134 (long-acting) is the utilisation of the extended plasma half-life of human serum albumin to achieve an extended therapeutic half-life. Here we discuss the application of human serum albumin as a half-life extension technology for GLP-1 therapeutics and how further developments in recombinant human albumin technology may further change the dosing paradigm.

**GLP-1 HALF-LIFE EXTENSION USING HUMAN SERUM ALBUMIN**

Human serum albumin is the most abundant plasma protein that has a number of interesting physicochemical properties that can be used in a pharmaceutical context. In particular, it has been demonstrated that therapeutic candidates can be attached to the protein can take advantage of the naturally extended half-life (approximately 19 days) of this protein to avoid rapid clearance from the body. Liraglutide, the short-acting GLP-1 analogue was the first of the GLP-1 analogues to reach the market using human serum albumin as a half-life extension technology. This molecule contained a short fatty acid “tag” attached to glutamic acid at position 26 on the peptide. Once in the plasma this “tag” can reversibly associate with fatty acid binding sites on circulating human serum albumin, preventing rapid renal clearance. This peptide had a half-life of around 11-15 hours and was the first once-daily treatment using a GLP-1 peptide. The next generation of GLP-1 medications are targeting once weekly dosing to both improve patient compliance and efficacy of the therapy. In the context of albumin-based therapeutics this concept has been demonstrated in the clinic by both albiglutide (GlaasmoSmithKline, London, UK), which is in Phase III trials, and CJC-1134 (Conjuchem, Los Angeles, CA, US), which in Phase II trials. These products use a covalent attachment of the GLP-1 peptide to the albumin molecule and achieve significant increase in half-life compared with the in vivo association used by liraglutide. This covalent attachment of the therapeutic target is achieved by either genetic fusion or chemical conjugation of the peptide to the albumin.

GSK’s albiglutide was developed in collaboration with Human Genome Sciences (Rockville, MD, US), which GSK acquired earlier this year, and uses albumin fusion technology licensed from Novozymes Biopharma (Figure 3). The process of albumin fusion involves the insertion of a contiguous piece of DNA that encodes for both the albumin and the GLP-1 peptide into a yeast-based expression system. Using this technology, a functional protein is expressed that has both the properties of albumin and the GLP-1 peptide.

In contrast, Conjuchem’s CJC-1134 is an engineered GLP-1 peptide, synthesised with an albumin binding group attached to a short linker molecule from the N-terminus of the peptide. This albumin binding group is then reacted with the free cysteine residue on the albumin molecule to form the conjugate. Despite these two methods of attachment being technically very different in terms of the product design, they both achieve very similar results clinically in terms of half-life and efficacy. The decision of whether to choose a fusion or conjugation route can be based on many different factors and both the albumin fusion and conjugates have their own benefits. For example, CJC-1134 uses a peptide containing a non-natural amino acid and would not be amenable a fusion route using the existing technology.

**Figure 1: Summary of current and developmental GLP-1 analogues utilising human serum albumin for half-life extension.**

<table>
<thead>
<tr>
<th>Product</th>
<th>Half-Life (h)</th>
<th>Dosing</th>
<th>Status</th>
<th>Product</th>
<th>Half-Life</th>
<th>Dosing</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Exenatide</td>
<td>2.4</td>
<td>Twice Daily</td>
<td>Approved</td>
<td>Bydureon</td>
<td>2.4 hours</td>
<td>Once weekly</td>
<td>Approved</td>
</tr>
<tr>
<td>Liraglutide</td>
<td>Nov-15</td>
<td>Once Daily</td>
<td>Approved</td>
<td>Albiglutide</td>
<td>6-8 Days</td>
<td>Once weekly</td>
<td>Phase III</td>
</tr>
<tr>
<td>CJC-1134</td>
<td>6-8 Days</td>
<td>Once weekly</td>
<td>Phase II</td>
<td></td>
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<td></td>
<td></td>
</tr>
</tbody>
</table>

**Figure 2: A schematic of the FcRn mediated albumin recycling process.**
the impact of this process and albumins half-life degradation is also one that has the therapeutic potential. It was considered that an understanding of albumin recycling is rescued from intracellular degradation is important. An albumin that is bound to the FcRn receptor is taken back to the surface of the cell and released back into the circulation. The FcRn recycling system is saturated and as a consequence not all albumins contained within any endosome will be recycled. If the albumin lost to intracellular degradation is also one that has the therapeutic molecule attached then this will also be degraded. It was considered that an understanding of the interaction between albumin and FcRn and the impact of this process and albumins half-life may ultimately lead to the ability to design therapeutics with designed half-life.

Novozymes Biopharma, in collaboration with scientists at the University of Oslo, have identified specific regions within the structure of the albumin molecule that are important for albumin FcRn binding. Subsequently, numerous albumin variants have been generated with single amino acid substitutions that display both increased and decreased binding to the FcRn receptor. A number of the variants have been tested in animal PK studies where a correlation between FcRn binding affinity and albumin half-life has been established.

In particular, one high-affinity variant demonstrated double the half-life of native sequence human serum albumin in rodent models. Ultimately, these changes in albumin half-life will translate to the therapeutic target and allow the drug development scientist to control the half-life of a target protein. In the context of GLP-1 peptides this technology could allow the shift from weekly, to every two weeks, to monthly dosing. To maintain the full flexibility of the application of this technology Novozymes has applied its extensive experience in recombinant albumin manufacture to ensure the drug development scientist to control the half-life of a target protein.

SUMMARY

GLP-1 analogues have become an important class of therapeutics in the treatment of type 2 diabetes, offering an alternative therapy where oral diabetic medications have failed. A major factor in the translation of this peptide from an un-druggable natural peptide to successful therapeutics is the application of human serum albumin as a carrier molecule. In particular, the development and expected launch of albiglutide, the first commercial albumin fusion therapeutic will only further enhance the growing pedigrees of this technology. The success with GLP-1 is likely to lead to the technology being applied with other therapeutic candidates in the anti-diabetic field.

To ensure that albumin half-life extension technology continues to meet the demands of innovative drug design, Novozymes has developed the next generation of albumin half-life extension technology. These albumin “variants” will open the door to longer dosing regimens for peptides such as GLP-1.

REFERENCES

Overlooked something?

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