Oral Diabetes Treatment

Taking the Needle out of Diabetes

Curtin University and Epichem Pty Ltd

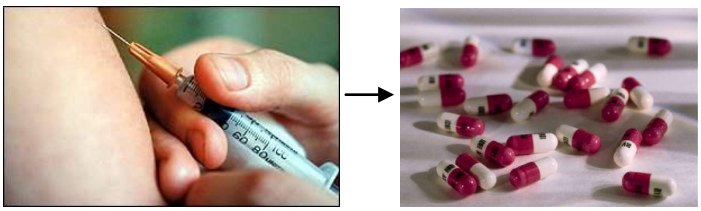
Curtin University and Epichem Pty Ltd are partners in a joint venture agreement to develop a new orally available drug for diabetics. If successfully developed, this drug could literally “take the needle out of diabetes”. It could be of particular utility as a front-line therapy for type 2 diabetics to delay the onset of insulin-dependency or could be taken conveniently by mouth at meal times to help control blood sugar levels of diabetics. It would be cheaper, non-invasive, suitable for distribution in emerging markets, and would rapidly penetrate the multi-billion dollar market for antidiabetic drugs

Market opportunity

Diabetes is a disease of epidemic proportion, affecting 6% of the world's population and representing 12% of total global healthcare expenditure. In 2009, the International Diabetes Foundation (IDF) predicted that the number of people with diabetes will balloon from 285M to 438M by 2030, a growth of 54% percent. In 2010, diabetes medications alone conservatively cost over $100 billion globally. Insulin sales alone exceeded $14 billion.

The need for the non-invasive delivery of insulin or a drug with insulin-like properties still remains. The drug under development in this project is a small molecule that will be delivered orally to trigger the same effects as insulin itself. Such a treatment for diabetics will have a number of sustainable competitive advantages over insulin therapy:

- *convenient oral administration leading to higher compliance with the treatment regime and consequently lower probability of complications of diabetes;*
- *circumvent the need for sterile needles and syringes and the complications and inconvenience associated with injections; and*
- *cheaper to manufacture, store, distribute and deliver.*



Orally available drug takes the needle out of diabetes.

Project description

Compounds with insulin-like properties have been discovered using 3D molecular maps (pharmacophores) to interrogate large pharmaceutical libraries for leads. Second generation compounds have now been developed based on these leads. Our current lead, EPL-BQ70, triggers the same cascade of events as insulin as it:

- *binds to and activates insulin receptors with micromolar potency;*
- *promotes specific phosphorylation of insulin receptors (IR Sure Fire ® / Alpha screen ® pY1150 & pY1151);*
- *promotes specific phosphorylation of Akt (Akt Sure Fire ® / Alpha screen ® pS473);*
- *is highly specific - activates insulin receptors but not insulin-like growth factor-1 receptors over the concentrations tested;*
- *lowers blood glucose in rats in a time-dependant manner and with an insulin-like profile;*
- *is as efficacious as insulin in vitro and in vivo;*
- *is a very drugable compound that:-*
  - *has good in silico ADME toxicity profiles*
  - *satisfys Lipinski's Rule of 5 (1 violation)*
  - *has low probability of cardiotoxicity*
  - *has low probability of hepatotoxicity*
  - *has low probability of CNS toxicity*
  - *is a small molecule (MW<500)*
  - *is easily synthesised in 4 steps*

Commercial application

An orally administered drug that mimics insulin could be of potential utility for all diabetics. It could be of particular utility as a front-line therapy for type 2 diabetics to delay the onset of insulin-dependency, an escalation of the disease that affects up to 27% of all type-2 diabetics. An orally available drug could also be of great utility about meal time, being taken conveniently by mouth with the meal, thus circumventing the need for additional injections using a rapid acting insulin. An insulin mimetic could also be of particular utility in the rapidly emerging markets in developing countries where poor infrastructure and health care systems make the administration of an injectable hormone drug difficult.

Competition

Diabetes: There is significant competition with most attention placed on non-invasive delivery of insulin. Strategies include pumps, inhalers, nasal sprays, patches and capsules. All have their limitations. Other approaches, transplantation of the pancreas and the insertion / cell graft of islet cells, have considerably more risk.

The most promising developments in recent times are the insulin promoters and sensitisers, “the Type 2 drugs”. The shortcoming of these treatments is they cannot replace the effects of insulin.

Intellectual property

US Patent 6,933,272 “Use of non-peptidyl compounds for the treatment of insulin related ailments” was issued in the United States (expiry date of 22 September 2019). Curtin University is the sole applicant of the patent. Curtin signed a Joint Venture Agreement with Epichem Pty Ltd In October 2008 to further develop the background IP.

A new patent application will be made to protect our newly discovered, second generation compounds and their use.

Investment

We are currently seeking investors and partners to assist in the development of these “break-through” products for the treatment of diabetes.

Further information

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EPL-BQ70 lowers blood glucose levels in an insulin-like manner *in vivo*

Background: A large number of rodent-based animal models have been developed for the study of diabetes. Each has different traits of diabetes and insufficient characterisation of many models can cast doubt over any conclusions drawn, especially through extrapolation of the results obtained from these models to humans. A normoglycaemic animal model can also be used for testing potential oral hypoglycaemic agents. This method allows for the effect of a drug to be tested in a healthy animal with intact pancreatic activity. In this study, *in vivo* testing of our best lead candidates was undertaken using a method developed at Novo Nordisk, Denmark (Schaffer, L. *et al.* Assembly of high-affinity insulin receptor agonists and antagonists from peptide building blocks. *Proc Natl Acad Sci USA,* 2003, **100**, 4435-39). This method uses anaesthetized normoglycaemic rodents to reduce fluctuations in blood glucose levels that follows repeated handling of fully conscious animals due to the release of stress hormones that counter-regulate insulin. This model substantially reduces the number of animals needed to achieve the necessary statistical validity in the experiments, which is a key ethical criteria governing research on animals. Importantly, this approach also relieves the animals of any stress or pain during the procedure.

Study: Figure 1 illustrates the change in blood glucose following the intravenous administration of vehicle), human insulin, or EPL-BQ70 to anesthetized Wistar rats following the procedure of Schaffer. In brief, the rats were anaesthetized, compound injected into the tail vein at time zero and glucose in blood, collected by puncture of the capillary vessels in the tail tip, was monitored over time with a glucometer. Relative to the rats receiving the vehicle alone, changes in blood sugar levels of EPL-BQ70 were highly significant from 20 to 120 minutes but were indistinguishable 180 minutes following administration of compound. The blood glucose lowering action of EPL-BQ70 was indistinguishable from insulin for the first 60 minutes following administration, but returned to control levels more quickly than insulin.

Conclusion: Delivered intravenously at 33mg/kg EPL-BQ70 lowers blood sugar levels with an efficacy approaching that of insulin. Studies of the effect of EPL-BQ70 in animal models of diabetes and its duration of effect following oral dosing or oral delivery, relative to subcutaneous delivery of insulin, are planned.

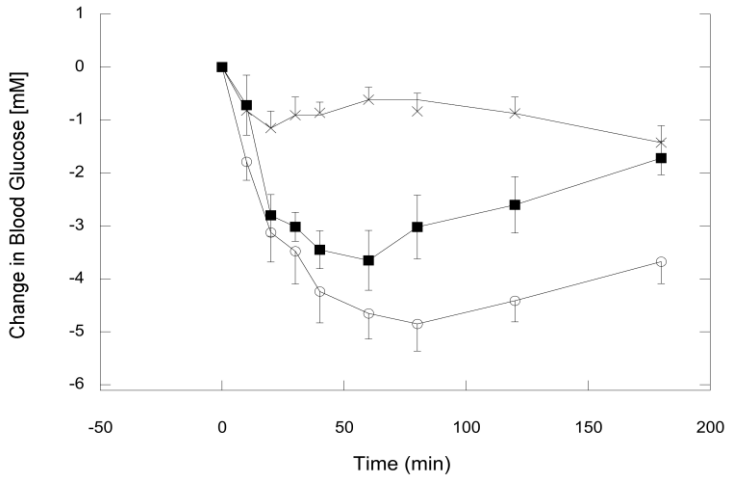

FIGURE 1: Blood glucose lowering comparison between EPL-BQ70 (33mg/kg or 72µM) and insulin (2.5nM) in a anaesthetised, normoglycaemic rats. Data are expressed as the mean plus or minus the standard deviation. Vehicle (x; *n* = 8 rats), human insulin (○; *n* = 8), or EPL-BQ70 (■*; n* = 8).

EPL-BQ70 specifically activates the insulin receptor with insulin-like efficacy

Background: Insulin relays its signal into cells by binding directly with the insulin receptor, which is a disulfide-linked heterotetrameric complex consisting of 2 extracellular alpha subunits and 2 intracellular beta subunits. Insulin binds to the alpha subunits and this induces phosphorylation of the tyrosine kinase domain on the beta subunits. This phosphorylation event is one of the earliest cellular responses to insulin and full kinase activation requires tyrosine phosphorylation of insulin receptor residues Tyr^{1146}, Tyr^{1150} and Tyr^{1151}. This in turn leads to the recruitment of SH2 and SH3 domain-containing proteins that act as signaling intermediates leading to the downstream activation of key targets such as Akt and ultimately to the downstream metabolic and mitogenic actions of insulin.

Insulin belongs to a family of growth factors including the very closely related insulin-like growth factors (IGF). Indeed insulin can bind with low affinity to the IGF receptor 1 (IGF-1R), which is also a tyrosine kinase that is widely expressed in many different cell types. Phosphorylation of residues Tyr^{1131}, Tyr^{1135} and Tyr^{1136} of the IGF-1 receptor is necessary for receptor activation. In this study, we also monitor these specific phosphorylations to ensure our compounds retain specificity for insulin and avoid the cancer potential of compounds that also stimulate the IGF-1 receptor.

Study: CHO cells overexpressing human insulin receptors or A431 cells expressing IGF receptors were treated for 12 min. at 37°C with either insulin, IGF-1 or EPL-BQ70 (Fig 2). Insulin promoted the specific phosphorylation of Tyr^{1150} and Tyr^{1151} of the insulin receptor with nM potency in CHO cells. Like insulin, EPL-BQ70 also promoted the specific phosphorylation of Tyr^{1150} and Tyr^{1151} of the insulin receptor with a potency of 170 µM and an efficacy similar to insulin. As expected insulin activated IGF-1 receptors in A431 cells, but with 100 fold less affinity relative to the sub-nM potency of IGF-1 on A431 cells. Like insulin, EPL-BQ70 specifically promoted the autophosphorylation of insulin receptors in CHO cells but failed to promote the autophosphorylation of IGF-1 receptors in A431 cells at the highest concentration (3 mM) tested.

Conclusion: EPL-BQ70 is a specific agonist of the insulin receptor.

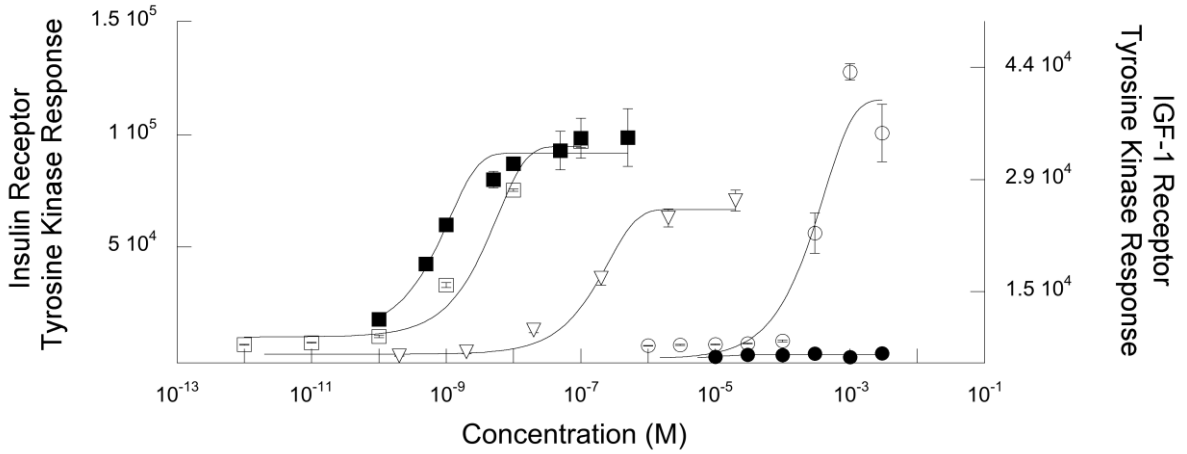

FIGURE 2: The autophosphorylation of Insulin and IGF-1 receptors by insulin, IGF-1 and EPL-BQ70**.** PerkinElmer's AlphaScreen technology and TGR Bioscience's *SureFire*™ assays were optimized as homogeneous cell-based assays for insulin and IGF-1 receptor phosphorylation. Stimulation of insulin receptors (phospho-Tyr 1150/1151) was measured in CHO cells with varying concentrations of insulin (□) or

EPL-BQ70 (○). This assay included a negative lysate prepared from confluent flasks of serum-starved Hela cells and a positive lysate prepared from confluent flasks of serum-starved Hela cells, treated with 200 µg/mL insulin for 5 min. Stimulation of IGF-1 receptors (phospho-Tyr1135/1136) was measured in A431 cells with varying concentrations of IGF-1 (■), insulin (▽) or EPL-BQ70 (●). This assay included a negative lysate prepared from confluent flasks of serum-starved A431 cells and a positive lysate prepared from confluent flasks of serum-starved A431 cells, treated with 10 µg/mL insulin for 15 min.

EPL-BQ70 specifically activates Akt (Protein Kinase B) with insulin-like efficacy

Background: The activation of the serine-threonine kinase Akt (also known as protein kinase B) is a key and central step to the actions of insulin. Akt also plays key roles in controlling survival and apoptosis. Akt activity is dependent on the phosphorylation of at least two key residues, Thr^{308} and Ser^{473}. The phosphorylation of the Ser^{473} is important for the phosphorylation of Thr^{308} and is necessary for the full activation of Akt.

Study: CHO cells overexpressing human insulin receptors were treated for 12 min. at 37°C with either insulin, or EPL-BQ70 (Fig 3). The cells were then lysed and the phosphorylation of Akt at Ser^{473} was measured using an AlphaScreen® SureFire® phospho-Akt 1/2/3 (Ser473) assay. As expected, insulin stimulated Akt with sub-nM potency. EPL-BQ70 also stimulated Akt in a dose-dependent manner with an efficacy similar to insulin and a potency of 160 µM (Fig. 3: open circles).

Conclusion: Like insulin, EPL-BQ70 activation of the insulin receptor leads to highly specific downstream Akt activation.

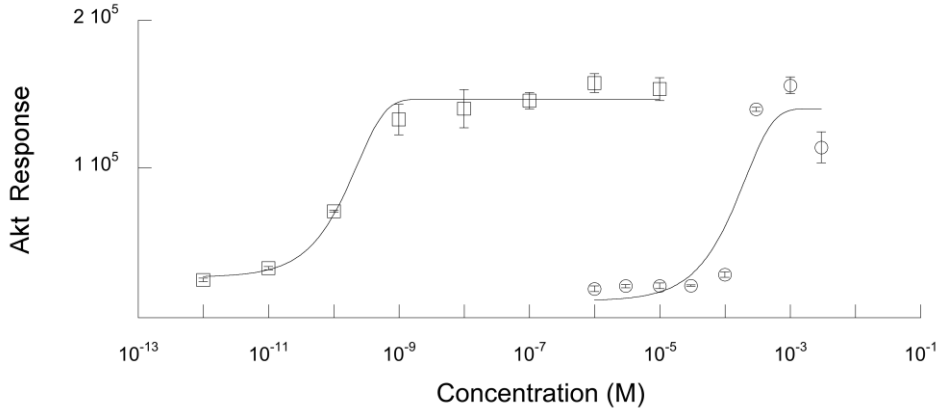

FIGURE 3: The specific phosphorylation of Akt (Ser^{473}) by insulin or EPL-BQ70. A PerkinElmer AlphaScreen and a TGR Bioscience's *SureFire*™ assay was optimized as homogeneous cell-based assays for the screening of specific Akt phosphorylation. Stimulation of Akt in CHO cells was measured with varying concentrations of insulin (□) or EPL-BQ70 (○). The AlphaScreen® SureFire® Akt (p-Ser^{473}) assay included a negative lysate prepared from serum starved HEK293 cells, treated with a combination of 2 mM wortamannin and 10mM LY294002 for 2 hours and a positive lysate prepared from serum starved HEK293 cells, treated with 20% serum for 15 minutes.

EPL-BQ70 shows no *in vitro* or *in vivo* toxicity in the current assays

Background: The *in vitro* phase of preclinical testing typically involves characterisation of the physiochemical properties of candidates, *in vitro* ADME and toxicity screens and advanced lead characterisation, which includes metabolite profiling. These predictive tools enable unsuitable new chemical entities to be terminated early in the research pipeline. Detailed *in vivo* ADME toxicity and metabolite profiling studies are undertaken following selection of a compound for potential clinical trial. Compounds with the potential to cause adverse events *in vivo* can be weeded out early in a drug discovery effort by a number of processes including *in silico* analysis, *in vitro* cytotoxicity screens and monitoring animals during and after *in vivo* efficacy testing.

Study: *In silico* analysis of EPL-BQ70 and several structurally related compounds suggest low probability of cardiotoxicity, hepatotoxicity or CNS toxicity. In addition, EPL-BQ70 and structurally related variants show no toxicity in L6 skeletal myoblast cell cultures, which are commonly used for *in vitro* toxicity testing of compounds. Furthermore no adverse events were recorded in rats (n=8) injected intravenously with EPL-BQ70 and the related structural variant EPL-BQ45 at concentrations up to 30mg/kg. Breathing, heart rate and behaviour of rats treated with EPL-BQ70 were indistinguishable from rats treated with vehicle alone. Autopsy of animals treated with EPL-BQ70 showed no obvious inflammation or damage to mucosal surfaces of the oesophagus, stomach, jejunum, sigmoid colon and rectum. No gastrointestinal hemorrhaging or bleeding within the eye suggests that vasculator was unaffected by EPL-BQ70.

Conclusion: Based on its physiochemical properties, cell culture and limited *in vivo* studies, EPL-BQ70 has a low probability of toxicity.

Physiochemical properties and descriptors of the lead compound (EPL-BQ70)

Background: In earlier studies, IM140 was identified as an insulin receptor agonist and lead compound for the oral treatment of diabetes (U.S. Patent 6,933,272 Use of non-peptidyl compounds for the treatment of insulin related ailments, 2005). IM140 was shown to: compete with insulin for binding to the insulin receptor; to activate (autophosphorylate) the insulin receptor; to promote glucose uptake in 3T3-L1 adipocyte cells and to lower blood glucose levels in a dose-dependant manner in severely hyperglycaemic, streptozotocin diabetic mice. However, IM140 was not in itself a good drug candidate. It synthesis was low yielding, requiring over 20 steps, and its physiochemical and predicted ADME properties were also less than ideal. Furthermore, its efficacy *in vitro* and *in vivo* was only about 30% that of insulin.

Study: Over 100 variants and several generations of compounds related to IM140 were synthesized and tested in a variety of biological assays. EPL-BQ70 was developed as the next generation drug lead. In contrast to IM140, EPL-BQ70 has many of the physiochemical characteristics of a good drug molecule with no significant violations in standard *in silico* ADME and toxicity screens. EPL-BQ70 has a molecular weight of less than 500 and is easily synthesized in just a few steps. The chemical scaffold of EPL-BQ70 is readily amenable to detailed structure activity relationship studies.

Conclusion: EPL-BQ70 has many of the physiochemical characteristics of a good drug molecule and a chemical scaffold that is readily amenable to a detailed structure-activity relationship study.