TrabiORAL™ – PHASE II

After a hiatus of few years and realising limitations in the adequate and optimal delivery of Insulin using Vitamin B12 as a carrier, our scientists at Transgene have identified a unique transporter system in the year 2011 in the intestine of mammals that is present in humans too.

Before proceeding further, number of in-vitro assays were carried out including:

1. Visualization and characterization of ligands present across the GI tract using fluorescent tagging
2. Cell permeability assays using CaCo2 and MTR 2 cell lines
3. Gut loop assays to determine the binding and uptake of orally administered protein and ligand bound protein moieties across the GI tract

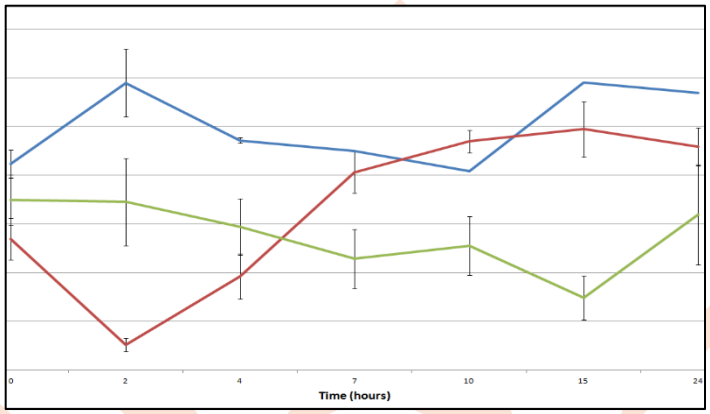
After identifying and characterization of the ligand Transporter system, Transgene planned to utilize this for oral delivery of protein drugs starting with Insulin. We realized that uptake of Insulin and its analogues or other proteins possessing their own receptors can work in conjunction with their naturally occurring receptors, thereby increasing the efficiency of uptake into the systemic circulation.

In the case of Insulin, the uptake alone by its own naturally occurring receptors is limited in the upper GI Tract. This is because in the acidic pH, insulin tends to form hexamers or worse aggregates, which render it biologically inactive. In order for insulin to be presented to its receptors, insulin has to be in the monomeric form. Our novel encapsulation technology maintains Lispro molecules in the monomeric form thereby presenting it to the insulin receptors in a biologically active state. Moreover, the encapsulation technology protects insulin from proteolytic degradation.

The current technology combines two of the uptake mechanisms for insulin. Firstly, it protects insulin from proteolytic degradation in the harsh environment of the intestine and maintains it n the monomeric form. As a result, biologically active form of insulin is presented to its receptors. The second part of the technology, exploits another transport system identified in the small intestine. Modified insulin taken up by this system is independent of the naturally occurring transporter. The different locations of the two receptors results in an extended uptake of oral insulin, which in turn reflects prolonged hypoglycemic effect. Our scientists have developed several formulation systems employing a proprietary transporter system, forming a targeted nano-system lattice.

TrabiORAL™ - Insulin

- TrabiORAL-Insulin formulation demonstrated in several animal studies lowering of blood glucose levels comparable to SC injected Insulin in Streptozotocin (STZ)-induced diabetic rats.
- Our oral insulin formulation produced a gradual but very sustained glucose reduction commencing at 2 hrs after oral administration lasting upto 15 hours unlike SC injected Insulin that lowered the serum glucose levels within 15 mts from the time of injection lasting for about 2-3 hrs.



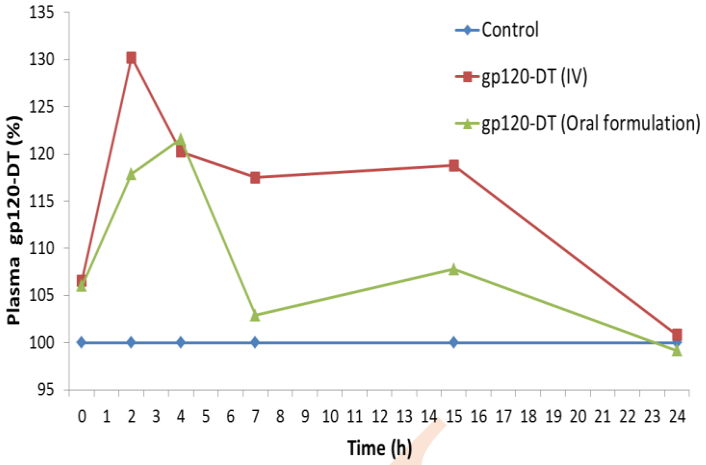
Summary:

- Utilization of identified ligand by Transgene has been well demonstrated in various studies on TrabiORAL - Insulin in STZ inducted diabetic animal models.
- Similar efficacy has also been demonstrated on other proteins much larger than Insulin such as fusion protein (68 kD) and Humira (150 kD).
- TrabiOral™ exploits the hSMVT carrier mediated and MCT1 systems for effective and safe transportation of the protein/peptide across the GI tract.
- Our technology, TrabiOral™, presents insulin or other proteins in their biologically active form to Receptors. A second mechanism exploits another unique transport system in the small intestine to our selected ligand. The modified insulin/protein taken up by this particular system is independent of the first transporter uptake mechanism mentioned above. The different locations of the two receptors results in an extended uptake of our oral insulin/protein formulation, which in turn reflects a prolonged hypoglycemic effect.

- TrabiOral, unlike several other oral delivery technologies chose to utilize transcytosis route and not paracellular pathways.
- TrabiOral exploits naturally occurring receptors for transport of protein or peptide of interest.
- The components of our formulation are all GRAS-approved ingredients which by themselves do not have any acute or chronic toxicity hence, no chronic adverse effects such as carcinogenicity or immunogenicity are expected.
- Apart from being a hypoglycemic agent insulin is also known to be a growth hormone. Frequent administration of insulin injections leads to the stimulation of adipocytes residing underneath the skin, in turn leading to collateral weight gain. Oral administration of insulin on the other hand, resembles a natural pathway that utilizes the first pass metabolism thereby entering into systemic circulation. The oral administration of insulin thus circumvents the mechanism that is responsible for weight gain.
- Our studies have indicated that each molecule might follow different route of entry in circulation which might be dependent upon characteristics of molecules too. To elaborate more, oral administration of insulin formulation lead to prolonged and sustained hypoglycemic effect however, low amount of insulin was detectable in serum whereas considerable amount of insulin was detected in spleen. This led us to hypothesize that insulin (5.8 kD) might be travelling through lymph and getting slowly released in serum. On contrary, in the case of orally administered formulation of fusion protein (68 kD), which lacks its own receptor in GI, appearance of fusion protein in serum was rapid and detectable.

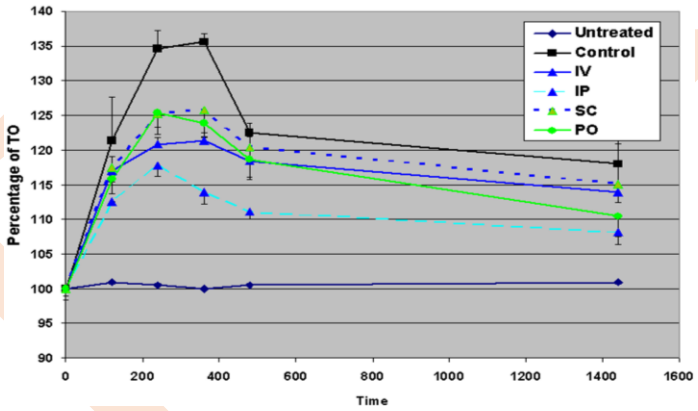
TrabiORAL™ - Synthesized (gp120-DT) Protein (68kDa)

We chose a Synthesized Protein because (1) it has NO naturally present Receptors of its own in the GI, (2) transport of the Synthesized Protein would ONLY be possible via Receptor-A. (Recall that in our Oral Insulin Studies 1 & 2, we believe transport occurred via BOTH receptors (Insulin + Receptor-A).



Conclusion: The detection of our orally administered Fusion protein in blood plasma successfully confirms the utility of Receptor-A for transport of any protein or peptide across the Gastro-Intestinal barrier.

TrabiORAL™ - Monoclonal antibody drug (Humira 150 kD)



Anti-inflammatory efficacy studies for oral anti TNF mAb formulation (solid green line) were carried out in Carrageenan treated mice. Anti-inflammatory efficacy of orally administered formulation was comparable to IV and sub cutaneous administration of mAb.

Final conclusion:

- We have successfully demonstrated a platform technology for peroral delivery of biotherapeutics, which can deliver a variety of therapeutic proteins ranging from 6kDa (insulin) to 150kDa (mAb's)s via the process of receptor mediated transcytosis.
- TrabiORALTM involves conjugation of biotherapeutics with bioactive ligands to facilitate delivery of biotherapeutics which lack their receptors in the GI tract.

- TrabiORALTM involves patented pH sensitive encapsulation technology which resists proteolysis in the acidic pH of the stomach and releases the biotherapeutic in alkaline pH of the intestine, thereby enabling access to intestinal receptors. As such, transport across intestinal barrier is independent of particle size.
- Utilization of more than one receptor systems for intestinal transport enables higher bioavailability, prolonged and sustained therapeutic activity and a flatter serum profile.

Drawback

The platform developed by TrabiORAL is proven to be sound in various studies conducted so far on different molecules of varying sizes. However, one important drawback observed by Transgene team is that of TrabiORAL™ inability to demonstrate adequate bio-availability of the chosen molecules including Insulin while demonstrating its efficacy as seen in the sustained reduction of serum glucose levels or in the case of other molecules such as fusion body of 68 kD and Humira of 150 kD. Therefore, the scientific team at Transgene has started its efforts to address this issue surrounding the lack of adequate bio-availability.