Production of Recombinant Human Insulin in Transgenic Safflower Seeds

Presented to:
ABIC
Aug 26, 2008
University College Cork, Ireland
History of Insulin

1922

THE INTERNAL SECRETION OF THE PANCREAS
BY F.G. BANTING, M.B., AND C.H. BEST, B.A.

The First Insulin Paper
The Journal of Laboratory and Clinical Medicine
7 (5) (February 1922): 465-480

1978

2008

Can we make enough insulin for everybody who needs it, at a price that is compatible with their means?
**Why plants?**

**Advantages of plant systems:**
- Higher capacity production and lower production costs for bulk protein
- Economical Manufacture of large volume products

**Seed-based expression systems:**
- Seeds have evolved as natural storage organs with high capacity for protein
- Low hydrolytic environment provides for stable storage
- Enables production to be decoupled from processing
**Testing and production vehicles**

**Arabidopsis**
- Well-characterized, model oilseed
- Small size and easily maintained for simplified production in growth facilities
- Easily transformed through floral dipping
- Rapid cycling:
  - Expression results in approximately 3 mos.
  - Sufficient protein for biochemical / functionality testing in 6 mos.

**Safflower**
- Safflower: *Carthamus tinctorius*, Family: Asteraceae
- Origin: Central Asia
- Semi-arid regions of North America, Australia and Asia

**Advantages for PMP production:**
- Low production acreages (<200,000 acres in N. America, forward contracted)
- No close weedy relatives found in the Americas
- Poor volunteer, low seed dormancy, low vegetative dispersal
Seed oilbodies

Cross-section of safflower seed

Oilbodies

Protein bodies

Oleosin
Phospholipids
Triacylglycerol
Oilbody
Oilbody-Oleosin Technology Platform

- Safflower
- Floret
- Seed
- Oleosin
- Proteins
- Oilbodies
- Nutritional Oils
Partitioning recombinant proteins using oilbodies

Coomassie Stained Protein Gel

Oleosins

rOleosin-FP

Coomassie Stained Protein Gel
Insulin
Meeting the Emerging Exploding Demand
Not Enough Insulin To Go Around

Diabetes Incidence: 2006

- **CAN/US**: 22.5 M
- **WEU**: 18.0 M
- **India**: 41.3 M
- **China**: 25.1 M

**Insulin Consumption**

- **Western World**: 70% Insulin Consumption
- **Rest of World**: 30% Insulin Consumption

Source: WHO, International Diabetes Federation
It’s Going To Get Worse

Diabetes Incidence: 2030

- **China**: 42.3 M
- **India**: 79.4 M
- **CAN/US**: 33.9 M
- **WEU**: 23.4 M

**Insulin Demand**
- **Western World**: 35% Insulin Demand
- **Rest of World**: 65% Insulin Demand

Source: WHO, International Diabetes Federation
Insulin Program Overview and Status
Insulin biosynthesis

1. Proinsulin is synthesized as a random coil on membrane-associated ribosomes.

2. The connecting sequence is cleaved to form the mature insulin molecule.

3. After membrane transport, the leader sequence is cleaved and the resulting proinsulin folds into a stable conformation.

4. Disulfide bonds form.

C-Peptide

Enzymatic Cleavage $\text{NH}_2$
**Expression construct**

**Construct**

- **insulin expression**: under β-phaseolin promoter/terminator
- **selectable marker**: *pat* gene under ubiquitin promoter/terminator for PPT resistance
- **transformation**: into *Agrobacterium* EHA101 then into the plant using the flower dipping method (Arabidopsis) or infection of explants (safflower)
- **post-extraction maturation of insulin**: functional insulin (5.7 kDa) generated by *in vitro* processing of fusion protein
Insulin expression in Arabidopsis

Coomassie blue stained gels and western blots (anti-insulin mab) of total seed protein
Insulin expression in safflower

[Image: A gel electrophoresis diagram showing the expression of insulin in Arabidopsis (WT) and 2 lines of safflower. The y-axis represents molecular weight in kDa, and the x-axis shows lanes labeled Arab. Insulin, Safflower Insulin Line A, and Safflower Insulin Line B. The gel indicates differential expression patterns across the lines.]
Plant-derived insulin:

- Is chemically equivalent to commercially-available human insulin
- Folds identically to commercially-available human insulin

Insulin - Chemical Equivalence
Plant-derived Insulin is properly folded

V8 protease fingerprinting

HPLC Run Time (min)

<table>
<thead>
<tr>
<th>Reference Insulin</th>
<th>Saflower Insulin</th>
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</thead>
<tbody>
<tr>
<td>Retention (min)</td>
<td>Mass (Da)</td>
</tr>
<tr>
<td>Fragment 1</td>
<td>27.73</td>
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<tr>
<td>Fragment 2</td>
<td>23.30</td>
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<tr>
<td>Fragment 3</td>
<td>22.52</td>
</tr>
</tbody>
</table>
Receptor Binding Assay

Graph showing the % Maximum Bound against Insulin (ng/ml) for three different insulins: Pharmaceutical RLD Insulin (red), USP Insulin (green), and Insulin (SemBioSys) (blue). The graph includes IC\textsubscript{50} values of 2.28, 2.08, and 2.10 ng/ml for each insulin type. Error bars are indicated as +/- SEM.
Rabbit Safflower Insulin Tolerance Test

Pharmacokinetics and Pharmacodynamics were indistinguishable from the reference drug.
Supplying World Insulin Demand

15,000 acres or 3 commercial farms

Supply for the entire planet in 2012

1 mile
Scale-up

~ 500kg seed

Pilot scale process
Insulin Process Development
Scalable, Cost-effective Solution

Safflower

Field of safflower

Fermentation vat

Insulin fermentation facility
Insulin Process Advantages
Regulatory Strategy: North America and Europe
Regulatory framework

- FDA and EMEA have both published guidelines on the manufacture of biologics in plants
- Plant-based systems will be expected to meet the standards of quality established for conventional production platforms (GMP starts in the field)
- Insulin Product Regulatory
  - EMEA published draft guidance on Biosimilar Insulin
  - FDA: insulin approved by CDER under FD&C Act. Therefore eligible for 505(b)(2) application
There is a clear regulatory process for safflower-derived insulin

Met with the FDA for a pre IND consultation in October 2006
Met with EMEA in May 2008

Conclusions from the FDA meeting:

• Insulin can follow the abbreviated 505(b)(2) rule
• First human trial will be a Phase II for pharmacokinetics & pharmacodynamics
  • 50 subjects, 1 month study
• Second human trial will be a Phase III for longer-term safety
  • 500 subjects, 6 months, 2 arms safflower insulin and Humulin®
  • 500 subjects, 6 months, 1 arm safflower insulin only
• No special regulations related to plant
Program Status

- **Completed** manufacture of sub-chronic toxicology batch
- Repeat dose (28-day) toxicology studies in rats and monkeys completed in-life phase Apr 20/08. All toxicology studies were “clean”
- IND submission **achieved in July 2008**
- Meeting and Briefing Book submission for EMEA SAWP took place May 2008
- Manufacture of Phase 1 trial batch **completion end Aug**
- Initiation of Phase 1 PK/PD clinical trial planned for Q3-Q4
- Phase III clinical trial planned for late 2009-2010 (12 month study)