Gamma-aminobutyric acid (GABA) treatment blocks inflammatory pathways and promotes survival and proliferation of pancreatic beta cells

Gérald J. Prud’homme, MD, FRCPC

Keenan Research Centre for Biomedical Science, St. Michael’s Hospital, Toronto. Department of Laboratory Medicine and Pathobiology, University of Toronto.
Email: prudhommeg@smh.ca
Limitations of current therapies of diabetes

Do not prevent or reverse type 1 diabetes (no cure).

Minimal or no improvement in the survival of pancreatic beta cells (type 1 or 2).

Do not induce replacement or regeneration of beta cells (type 1 or 2).
EFFECTS NEEDED TO CURE TYPE 1 DIABETES

• Stop the autoimmune (inflammatory) reaction that kills beta cells.
• Increase the resistance of beta cells to injury.
• Stimulate the regeneration of beta cells, or replace these cells.
• Research aspect: human beta cells are different from mouse. Drugs must work on human cells.
GLP-1: an incretin hormone with multiple direct effects on human physiology

- Pancreas: 
  - Glucose-dependent insulin secretion (β)
  - Insulin synthesis (β)
  - Glucose-dependent glucagon secretion (α)

- Intestine: 
  - L-cells secrete GLP-1
  - Degraded by DPP-4

- Liver: 
  - Glucose production

- Brain: 
  - Satiety

- Stomach: 
  - Gastric emptying

- Heart: 
  - Cardioprotection
  - Cardiac function

Adapted from Baggio & Drucker. Gastroenterol 2007;132:2131-57
GLP-1 receptor agonists

• Effective in the treatment of type 2 diabetes
• Several drugs available or under investigation: exenatide [Byetta], liraglutide, dulaglutide, etc.
• Not effective in type 1 diabetes (lack anti-inflammatory and regenerative capacity).
GABA
Gamma-aminobutyric acid
GABA is an inhibitory neurotransmitter in the brain, but also present in the pancreas.
GABA

**Brain:**
Major inhibitory neurotransmitter.

**Islets:**
Inhibits α cells, but stimulates β cells.

**Immune system:**
Inhibits lymphocytes and macrophages
GABA RECEPTORS

Type A (GABA-A receptor):
Fast acting ligand-gated chloride channel (many variants).
Blocked by picrotoxin.

Type B (GABA-B receptor):
Slow acting G-protein coupled receptor.
Blocked by saclofen.

Neurons and islet cells: Express both receptors.

Lymphocytes: Type A only.
GABA suppresses proliferation of human T cells (anti-CD3 antibody)
## Immunotherapeutic effects of GABA in T1D

<table>
<thead>
<tr>
<th>Strain/model</th>
<th>Immune effects</th>
<th>Disease course</th>
</tr>
</thead>
<tbody>
<tr>
<td>NOD(prediabetic)</td>
<td>Th1 cells↓, IFN-γ↓ IL-12↓, Treg↑</td>
<td>prevented diabetes (insulitis↓)</td>
</tr>
<tr>
<td>NOD (diabetic)</td>
<td></td>
<td>reversed transiently</td>
</tr>
<tr>
<td>NOD-TCR8.3</td>
<td>CTL response↓</td>
<td>prevented (insulitis↓)</td>
</tr>
<tr>
<td>CD1 mice (low dose STZ, diabetic)</td>
<td>IL-1↓, TNF-α↓ IL-12↓, IFN-γ↓</td>
<td>reversed diabetes (insulitis↓, β-cell regeneration)</td>
</tr>
</tbody>
</table>

**Reviewed in:** Prud’homme et al. Autoimmunity reviews, 2015, 14:1048-56.
Inhibition of NF-kB in Mouse T cells
(anti-CD3/CD28 stimulated)
GABA suppresses activation of NF-κB in human islets through GABA-A receptors
GABA BLOCKS AUTOIMMUNE REACTION AGAINST BETA CELLS
GABA induces beta-cell regeneration
Mouse:

GABA prevents β-cell death, stimulates β-cell proliferation, increases insulin secretion, and promotes β-cell regeneration.

GABA exerts anti-inflammatory and immunosuppressive effects.

Humans: Similar to mouse??
GABA stimulates insulin secretion
Human beta cells in culture

![Graph showing the survival of human beta cells over days in culture with and without GABA treatment. The x-axis represents days in culture (0-8), and the y-axis represents live cells per sample. The graph shows a decline in cell count over time for both treated and non-treated samples, with GABA-treated cells showing a slight increase in survival compared to non-treated cells.]
GABA and Liraglutide (GLP-1R agonist) ameliorate human beta-cell survival (24 h in vitro; additive effect)
Non-treated islets

Ki67 - red

DAPI - green

merge
Islets treated with 100 μM GABA

Ki67 - red

DAPI - green

merge
The effect of GABA on the proliferation of the cell in human pancreatic islets treated in vitro for 19 h.

Ki67 staining,
See images below
Human islets transplanted into immunodeficient mice: GABA stimulates growth (regeneration) of beta cells
HYPOTHESES

1. Combined therapy with GABA and a DPP-4 inhibitor (DPP4-I) will improve protection of human beta cells against injury/apoptosis and induce regeneration.
2. A completely oral therapy will be effective, which is a major clinical advantage.
GABA AND GLP-1 WORK TOGETHER

• Our most recent data show that GABA and GLP-1 are more effective when administered together.
• Improved beta-cell survival and regeneration.
• Effective on human beta cells.
• Completely oral therapy protects against diabetes in experimental model.
GABA AND DPP-4I collaborate to increase beta-cell mass. Red = insulin; green = glucagon; blue = DAPI. Treatment with combined drugs was superior to induce proliferation (Ki-67+ cells) and reduce apoptosis (Tunel assay); data not shown.
GABA and GABAergic drugs (agonistic) increase SIRT1 expression in INS-1 beta-cell line
GABA increases Klotho (alpha-Klotho)

In humans Klotho normally declines with age, and is abnormally low in diabetic patients (type 1 and 2).

Klotho KO mice have multi-system disease and accelerated aging.

Klotho has multiple protective effects on beta cells. Importantly, it inhibits NF-kB activation and exerts anti-apoptotic effect.
Membrane-bound klotho binds FGFR1 and facilitates binding of FGF23 to this receptor. Soluble klotho acts as a hormone with the receptors yet to be characterized. KL1 and KL2 domains structurally resemble glucosidase, but their enzymatic activity is questionable.
Binding partners of α-klotho

- **FGF23, FGFR1**
  - Cell proliferation
  - Caspase-3 cleavage
  - Apoptosis

- **IGF-1**
  - α-klotho shedding (activation of mTOR)
  - IGF-1 tyrosine kinase
  - FOXO (antagonizes suppression by IGF-1)
  - MnSOD expression
  - Suppression of pancreatic cancer

- **TGF-βRII**
  - Prevents binding of TGFβ1 to TGF-βRII
  - Inhibits EMT

- **Wnt family members**
  - Inhibits accelerated senescence
  - Suppresses tumor development

- **?**
  - Multiple effects mediated by non-described receptors
KLOTHO INHIBITS NF-κB ACTIVATION

Buendia et al., Vitamins and Hormones, 2016, 101: 119-147
Schematic representation of the Klotho participating in intracellular signaling pathways. Klotho protein is involved in several intracellular signaling pathways that are essential for the regulation of many cellular processes, including aging and senescence.

From: M. Sopjani et al. Klotho and Intracellular Signaling Current Molecular Medicine, 2015, Vol. 15, No. 1 33
The level of circulating klotho is decreased in diabetic C57 mice. It is restored by GABA in drinking water.
The level of circulating klotho negatively correlates with the level of blood glucose.

<table>
<thead>
<tr>
<th></th>
<th>blood glucose, mM vs. non-treated</th>
<th>blood glucose, mM vs. GABA-treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pearson r r</td>
<td>-0.6855</td>
<td>-0.4399</td>
</tr>
<tr>
<td>95% confidence interval</td>
<td>-0.8771 to -0.3055</td>
<td>-0.7522 to 0.034</td>
</tr>
<tr>
<td>R squared</td>
<td>0.4699</td>
<td>0.1935</td>
</tr>
<tr>
<td>P value</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P (one-tailed)</td>
<td>0.0012</td>
<td>0.0339</td>
</tr>
<tr>
<td>P value summary</td>
<td>**</td>
<td>*</td>
</tr>
<tr>
<td>Significant? (alpha = 0.05)</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Number of XY Pairs</td>
<td>17</td>
<td>18</td>
</tr>
</tbody>
</table>
High Glucose induces release of Klotho by cultured human pancreatic islets.
Cultured human pancreatic islets release Klotho. The release is notably activated by muscimol and baclofen.
Soluble α-klotho increases viability of INS-1

Klotho knockdown
Recombinant Klotho Cell viability
Ability of GABA to increase cell viability and to protect INS-1 cells against STZ toxicity depends on α-klotho expression

Klotho knockdown
GABA
Cell viability
Effects of Klotho/our preliminary data:

1. Klotho is expressed by rat (INS-1) and human pancreatic cells (donor islets).

2. Cultured islet cells actively release soluble Klotho.

3. The level of circulating Klotho in mouse serum is severely decreased during STZ-induced diabetes. GABA, but not Liraglutide, restores it.

4. Ability of GABA to increase the survival of beta cells under stress depends on the expression of Klotho.
GABA in food and beverages: It is a safe natural compound
GABA INCREASES GROWTH HORMONE
SUMMARY: GABA has key effects against diabetes (studies performed with human cells)
1) It prevents beta-cell injury and death.
2) It promotes the regeneration of beta cells.
3) It suppresses immune cells that cause autoimmunity and beta-cell loss.
4) It increases SIRT1 and Klotho, which suppress NF-κB activation and exert protective effects on beta cells.

Future Goals

1. Clinical development of a new treatment for type 1 and 2 diabetes: GABA therapy, with or without GLP-1.
2. Determine whether GABA therapy has a role in the treatment of other chronic diseases.
Research Team
Dr. Gérald Prud’homme
Dr. Qinghua Wang
Dr. Tianru Jin
Dr. Yelena Glinka
Dr. Wenjuan Liu
Ms. Merve Kurt

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