The effect of Exenatide Therapy on inflammation, insulin requirement and weight in Obese Type 2 diabetes Mellitus patients on Insulin

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• Diabetes Mellitus along with its complications costs 174 billion Dollars (Economic Costs of Diabetes in the U.S. in 2007 Diabetes Care March 2008)

• GLP1 mimetic/analogues/DPP4 inhibitors – 
  ➢ New and effective adjunctive treatment strategy to manage Type 2 Diabetes Mellitus
GLP1 INCRETIN

STIMULATES INSULIN RELEASE

INHIBITS GLUCAGON RELEASE

DPP-4 INHIBITORS

BLOCKS DPP4 ENZYME TO DECREASE GLUCOSE

LOWER BLOOD SUGAR
GLP1

- L cells of jejunum/ileum

**Insulinotropic effect,**\(^8,9\)
INSULIN RELEASE IN PRESENCE OF HYPERGLYCEMIA

**Insulin biosynthesis** and gene expression\(^5,10\)

The transcription of glucokinase and the GLUT 2 transporter genes.\(^{11}\)

**GLUCAGONOSTIC EFFECT:**\(^8,18,19\)
- GLP1 R on alpha cells
- Insulin
- Somatostatin

GLP-1 receptor activation directly stimulates beta-cell replication and neogenesis\(^{14-16}\)
(RODENT STUDIES)

**INHIBITS BETA CELL APOPTOSIS**
animal models of obesity and hyperglycemia.\(^{17}\)
EXENATIDE
Synthetic exendin-4

- Exenatide exhibits actions that are similar to those of GLP-1
- Stimulation of insulin secretion only when blood glucose concentrations are elevated
- Suppression of postprandial glucagon secretion. BUT DOES Not impair normal glucagon response to hypoglycemia
- Restoration of First-phase of insulin response
- Slowing of gastric emptying and promotes satiety (5, 6)
Obesity

Diabetes Mellitus

Insulin Resistance

- Pro inflammatory
- High oxidative state $^{48-56}$

IL6/CRP/TNF

ALPHA $^{57}$

Insulin IV
DIET INDUCED WEIGHT LOSS

• TNF ALPHA
• IL6
• CRP$^{52,76-81}$

CALORIE RESTRICTION

• OXIDATIVE STRESS
• INFLAMMATORY MEDIATORS$^{49,52,82}$
Two cardinal indices of inflammation at the cellular level

- Glucose and mixed meal\textsuperscript{63-65}

\[ \text{NFkB} \] \quad \downarrow \quad \text{Total cellular } \text{IkB}\alpha

\[ \text{NFkB} \] \quad \uparrow
**RATIONALE**

- **Obesity and Diabetes**
  - States of *increased inflammation*
  - Exenatide is expected to lead to *decreased inflammation* by virtue of *better glycemic control* and *weight loss*.

- Exenatide results in better control of T2 Diabetes Mellitus

- Long term use of exenatide reduces insulin requirement.
AIMS

• Exenatide has anti inflammatory effect
  - Single dose of Exenatide causes anti inflammatory effect
• Effect of exenatide over 12 weeks v/s placebo on
  - HbA1c
  - Fasting blood glucose
  - Body weight
  - Total Insulin Requirement
Single-center, randomized, placebo-control, single blinded (patient) prospective study.

N=24 subjects
Type2DM
On insulin

PLACEBO
N=12

EXENATIDE
N=12
Inclusion criteria

• Males or females 20-75 years of age inclusive.
• Type 2 diabetes
• On insulin therapy
• HbA1c 7.5% and ≤ 10.0%
• BMI ≥ 30 kg/m²
• Subjects on statins, ACE inhibitors, metformin and will be allowed as long as they are on stable doses of these compounds and the dosage is not changed during the study.
Exclusion criteria

- Coronary event or procedure (myocardial infarction, unstable angina, coronary artery bypass surgery or coronary angioplasty) in the previous four weeks
- Pregnancy
- Hepatic disease (abnormal LFT’s)
- Use of DPP4 inhibitors.
- Renal impairment (serum creatinine > 1.5)
- Participation in any other concurrent clinical trial
- Any other life-threatening, non-cardiac disease
- Uncontrolled hypertension (BP > 160/100 mm of Hg)
- Use of an investigational agent or therapeutic regimen within 30 days of study
• Dietitian/Certified Diabetes Educator meeting on Day 0

• Dietary recommendations - American Diabetes Association guidelines

• They were randomized to receive either
  • **Exenatide 10mcg or Placebo** 30 min before breakfast and dinner for 12wk.

• The dose of Exenatide was started at
  **5mcg twice daily for 1 wk to ensure tolerability.**
24 obese Type 2 diabetes HbA1c(7.5 --9%)

- All patients were on Insulin
- Stable doses of antidiabetic medications
- Stable weight over prior 4 weeks
- All were on 1-2g of Metformin

- Statins/ACEI DOSES were not changed during the study
- No patient was on Thiazolidinediones, Antioxidants, NSAIDS.

- 14/24: sulfonylureas glyburide/ glipizide 5–10 mg/d).
- 10/24: long acting insulin (glargine/detemir)
- 12/24: long acting + pre meal bolus
- 2/24: Novolog 70/30 BID
12 WEEK study

Single dose study

exenitide 5micg

placebo

0 day  3wk  6 wk  12 wk

0hr  2hr  4hr  6hr
Insulin titration

- Target fasting blood glucose 100
- 2 hour post prandial 160

wk3

wk6
• Mono Nuclear Cells collection- Na EDTA-washed Hanks salt solution- yeild 95%
• Reactive Oxygen Species generation: measured by chemiluminiscence
• Nuclear NFkB and Oct-1 DNA-binding activity was measured by EMSA (electrophoretic mobility shift assay)
• Nuclear extracts -salt extraction method from MNC
Real Time-PCR.

Quantification of JNK-1, TLR-2, TLR-4, TNF alpha

- The mRNA expression of JNK-1, TLR-2, TLR-4, TNF alpha, SOCS-3, IL-1B, and IL-10
Statistical analysis was conducted using Sigma Stat software (SPSS Inc., Chicago, IL)

• All data are represented as mean ± SE. Baseline measurements were normalized to 100%, and changes from baseline were calculated as percent change from baseline.

• Statistical analysis was carried out using one way repeated measures ANOVA (RMANOVA) with Holm-Sidak post hoc test.

• Two-factor RMANOVA followed by Dunnett’s post hoc was used for multiple comparisons between different treatments.

• Paired t test and Student’s t test were used where appropriate.

• Multivariate analysis of changes in inflammatory mediators from baseline with changes in free fatty acids (FFA), insulin, glucose, percent HbA1c, body mass index, Systolic and diastolic blood pressure was performed using multiple linear regression.
<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>At 12 Wk</th>
<th>Exenatide</th>
<th>Baseline</th>
<th>At 12 Wk</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (Yrs)</strong></td>
<td>54±4</td>
<td></td>
<td>56±3</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Weight (lbs.)</strong></td>
<td>231±13</td>
<td>234±18</td>
<td>251±18</td>
<td>251±20</td>
<td></td>
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<tr>
<td><strong>BMI(Kg/m²)</strong></td>
<td>39.1±1.6</td>
<td>39.1±1.7</td>
<td>39.8±2.0</td>
<td>39.2±1.8</td>
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<tr>
<td><strong>HbA1c(%)</strong></td>
<td>8.5±0.3</td>
<td>8.0±0.3</td>
<td>8.6±0.4</td>
<td>7.4±0.5</td>
<td>Â</td>
</tr>
<tr>
<td><strong>Diabetes duration (Yr)</strong></td>
<td>12±2</td>
<td></td>
<td>12±2</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Fasting glucose (mg/dl)</strong></td>
<td>128±13</td>
<td>139±33</td>
<td>139±17</td>
<td>110±9</td>
<td>Â</td>
</tr>
<tr>
<td><strong>Fasting insulin(uU/ml)</strong></td>
<td>13.1±3.1</td>
<td>13.9±5.9</td>
<td>12.7±2.8</td>
<td>16.4±3.2</td>
<td>Â</td>
</tr>
<tr>
<td><strong>FFA (mM)</strong></td>
<td>0.64±0.08</td>
<td>0.61±0.09</td>
<td>0.69±0.07</td>
<td>0.50±0.03</td>
<td>Â</td>
</tr>
<tr>
<td><strong>SBP (mmHg)</strong></td>
<td>128±5</td>
<td>130±6</td>
<td>134±6</td>
<td>127±5</td>
<td></td>
</tr>
<tr>
<td><strong>DBP (mmHg)</strong></td>
<td>78±2</td>
<td>76±4</td>
<td>82±2</td>
<td>77±3</td>
<td></td>
</tr>
<tr>
<td><strong>Insulin Dose (U)</strong></td>
<td>82±13</td>
<td>88±13</td>
<td>105±30</td>
<td>105±31</td>
<td></td>
</tr>
</tbody>
</table>
• Data represented as mean ± Standard Error (SE)

• Å - P value <0.05 (paired t test compared with baseline)
Fasting blood glucose fell from 139 ±17 to 110 ±9 mg/dl (P<0.05)
HbA1c from 8.6% ± 0.4% to 7.4%±0.5%(P<0.05)

Data are presented as mean +/- SE; n =12 each. * and **, P < 0.05 by RMANOVA (compared with baseline) in exenatide and placebo groups, respectively; # P < 0.05 by two-way RMANOVA compared with control groups.
Insulin increased (P < 0.05) in the Exenatide group whereas it did not change significantly in the placebo group.

Data are presented as mean ±SE; n = 12 each. * and **, P # 0.05 by RMANOVA (compared with baseline) in exenatide and placebo groups, respectively; # P < 0.05 by two-way RMANOVA compared with control groups.
Percent change in FFA after placebo and exenatide 10 mic g twice daily for 12 wk

Percentage change in FFA (D) after a single dose of 5 mic g exenatide or placebo in type 2 diabetic subjects.

Percentage FFA decreased by 21.5% from baseline (P<0.05) with exenatide.

Data are presented as mean +/- SE; n =12 each. * and **, P # 0.05 by RMANOVA (compared with baseline) in exenatide and placebo groups, respectively; # P < 0.05 by two-way RMANOVA compared with control groups.
Percent change in ROS generation by MNC after
(A) Placebo and exenatide 10 micg bid for 12 wk
(B) Single dose of placebo or exenatide (5 micg) and after 6 h
Change in NFkB/Oct-1 DNA-binding activity (B and C)

Data are presented as mean +/-SE; n = 12 each. *, P < 0.05 by RMANOVA (compared with baseline); #, P < 0.05 by two-way RMANOVA compared with control groups.
Change of mRNA expression of TNFalpha (D and E)

IL-1B (F and G) from baseline (100%) after placebo and exenatide 10 micg twice-daily treatment for 12 wk and after 6 h of a single dose of placebo or exenatide (5 micg) in type 2 diabetic subjects.
• Percent change in JNK-1 (B), TLR-2 (C), and SOCS-3 (D) proteins in MNC after placebo and exenatide 10 micg twice-daily treatment for 12 wk (W) in type 2 diabetic subjects.

Data are presented as mean +/-SE; n =12 each. *, P <0.05 by RMANOVA (compared with baseline); #, P <0.05 by two-way RMANOVA compared with control
Discussion

- **Data shows** clearly that exenatide **suppresses several indices of inflammation** when given over a period of 12wk.
- They include ROS generation by MNC,
  - Intranuclear NFkB binding,
  - Expression of TNF alpha, JNK-1, TLR-2, IL-1B, and SOCS-3 in MNC
  - There was Fall in the plasma concentrations of MCP-1, SAA, IL-6, and MMP-9
- All these changes were independent of weight loss
• Effect more impressive as all patients were on insulin which has its own anti-inflammatory effect

• An explanation for the lack of exenatide induced weight loss could be the short duration of our study in subjects on relatively large doses of insulin.

• HbA1c was also reduced significantly from 8.6 to 7.4%, and the reduction in calorie loss from glycosuria could have neutralized the effect of exenatide on weight loss.
Potent but transient Rapid Anti inflammatory effect: single injection of 5micg exenatide: peak effect at 2hrs : coincides with time of peak plasma concentration.
• Possible mechanisms for the anti-inflammatory/antioxidant effects of exenatide include the
  ➢ suppression of FFA £
  ➢ enhancement of the anti-inflammatory action of insulin £
  ➢ Suppression of Glucagon (not studied)
Limitations of study

• Small sample size
• Diet recommendations were made at baseline and dietary history was not collected at the end of the study.
• Likely that subjects did not make any substantial dietary changes during the course of the study.
Conclusion

Exenatide when administered for 12 wk

- comprehensive ROS suppressive and anti-inflammatory effect in presence of insulin

- Single dose of 5μcg: rapid but transient anti-inflammatory effect.
Future studies

• Whether exenatide might be used in acute inflammatory settings in the intensive care unit or following heart attacks and strokes, where a rapid anti-inflammatory effect is required and such drugs may be of potential use.
• THANK YOU
Acknowledgement

• Faculty Diabetic Research Center of WNY


