Pharmacologic activation of the cholinergic anti-inflammatory pathway reverses obesity-induced insulin resistance

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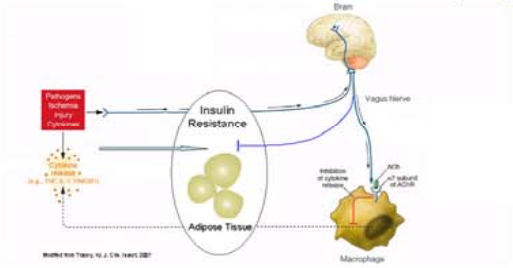
Abstract

Introduction: The incidence of metabolic syndrome is significantly increasing in the United States. TNF and other proinflammatory cytokines have been implicated in the pathogenesis of obesity-related insulin resistance. TNF production is controlled by a neural circuit termed the cholinergic anti-inflammatory pathway. Signals transmitted by the vagus nerve suppress TNF release through an alpha7 nicotinic acetylcholine receptor subunit dependent mechanism. We postulated that activating the cholinergic anti-inflammatory pathway by administration of a selective alpha7 nAChR agonist, GTS-21, would reduce TNF-induced insulin resistance and cardiovascular risk in rats fed a high fat diet (HFD).
Methods: Rats were fed standard chow, or chow with 10% lard for three weeks. Rats were then treated with GTS-21 (4mg/kg subcut bid) or saline for 1 week. Insulin resistance was measured using an intravenous insulin tolerance test (ITT). Blood samples were taken for measurement of CRP and HDL cholesterol levels
Results: As expected, rats fed a high fat diet for three weeks developed insulin resistance (KITT control 7.42±0.63 %/minute vs KITT HFD 5.09±0.5, n = 8, 9 respectively, p<0.01). Treatment with GTS-21 led to an improvement in insulin sensitivity (KITT saline 4.11±0.49 %/minute, KITT GTS-21 5.15±0.34, n = 6, 5 respectively). Animals treated with GTS-21 also had elevated levels of HDL cholesterol.
Conclusions: These results indicate that activation of the cholinergic anti-inflammatory pathway by administration of GTS-21 can improve insulin sensitivity, and may decrease cardiovascular risk.

Introduction

- Prevalence of obesity in the US exceeds 30% of the population
- Obesity is associated with significant morbidity
  - Type 2 diabetes mellitus
  - Cardiovascular disease
- Type 2 diabetes = insulin resistance + impaired insulin secretion
- Obesity leads to diabetes largely by inducing insulin resistance
- Obesity → ↑ pro-inflammatory cytokines → insulin resistance
- Activation of the cholinergic anti-inflammatory pathway suppresses production of proinflammatory cytokines
- GTS-21 is a selective α7 nicotinic acetylcholine receptor agonist



Hypothesis: GTS-21 treatment will reverse insulin resistance in obese rats

Objective

To investigate the effect of pharmacologic activation of the cholinergic anti-inflammatory pathway on insulin resistance and associated cardiovascular risk profile in obese rats

Methods

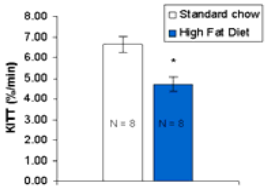
1. Highly Palatable Diet: standard chow + %10 lard = 33% Fat, 22% Protein, 45% Carbohydrates
2. Saline or GTS-21 0-4 mg/kg subcut bid x 0-7 days

3. Insulin tolerance test, Calculate K_{ITT} $(0.693/t_{1/2}) \times 100$ = % decline in blood glucose per minute

4. Collected blood and tissue samples for analysis of cytokines via ELISA and insulin signaling molecules via immunoblot

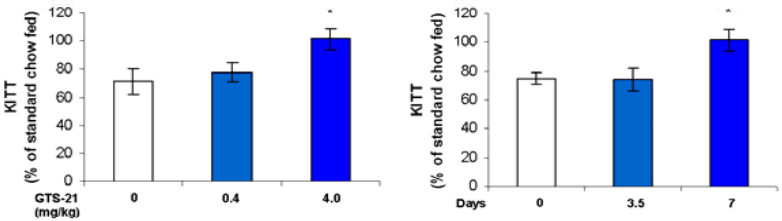
Results

High fat diet rapidly induces insulin resistance



Insulin sensitivity after two weeks of high fat diet (p < 0.01)

GTS-21 reverses high fat diet-induced insulin resistance

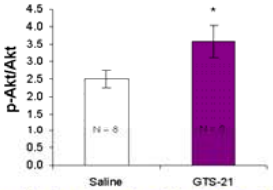


Insulin sensitivity in rats fed a high fat diet and treated with GTS-21 0-4 mg/kg for 0-7days (n=6-9 per group, * p < 0.01)

Metabolic Parameters

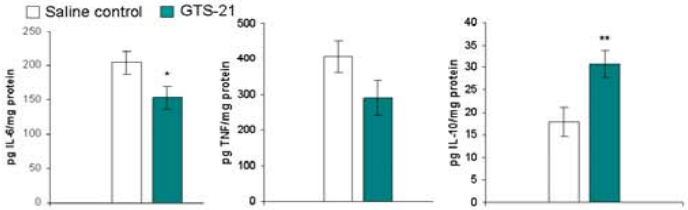
	Saline control	GTS-21 treated	P-value
Food Intake (g/rat/day)	**27.7 +/- 1.1**	**26.2 +/- 1.3**	**0.38**
Weight change during tx (g)	**18.3 +/- 3.0**	**13.4 +/- 2.9**	**0.25**
Fasting glucose (mg/dL)	**240 +/- 10**	**254 +/- 10**	**0.34**
Fasting insulin (pg/mL)	**630 +/- 77**	**420 +/- 38**	**<0.05**

GTS-21 enhances insulin signaling



Activated Akt (a key insulin signaling molecule) relative to total Akt in visceral adipose tissue of high fat- fed rats after GTS-21 (4mg/kg) or saline x 1 week (p < 0.05)

GTS-21 treatment decreases inflammation



Cytokine concentrations in visceral adipose tissue of rats fed a high fat diet and treated with GTS-21 (4mg/kg) or saline x 1 week (n= 8-10 per group, * p < 0.05, ** p < 0.01)

Cardiovascular risk markers

	Saline control	GTS-21 treated	P-value
CRP (mg/dL)	**< 0.05**	**< 0.05**	
PAI-1	**1.9 +/- 0.3**	**1.7 +/- 0.3**	**NS**
HDL (mg/dL)	**46.5 +/- 1.8**	**55.5 +/- 2.5**	**< 0.01**
TGF-ß (ng/mL)	**82 +/- 3.6**	**95 +/- 4.7**	**< 0.05**

Conclusions

- Pharmacologic activation of the cholinergic anti-inflammatory pathway reverses insulin resistance and improves cardiac risk in obese rats

Future Directions

- Evaluate effect of cholinergic stimulation on coronary disease in atherosclerosis prone rodents
- Extend these *in vivo* findings to human disease in clinical trials evaluating the effect of cholinergic agonists or acetylcholinesterase inhibitors in patients with type 2 diabetes

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