Effect of Emulin on Blood Glucose in Type 2 Diabetics

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ABSTRACT Emulin™ is a patented blend of chlorogenic acid, myricetin, and queretin that has shown efficacy in reducing midday and post-oral glucose tolerance test (OGTT) area under the curve (AUC) glucose in streptozotocin-treated rats. The purpose of this study was to determine if similar effects would be evident in type 2 diabetic humans. Forty human subjects with confirmed type 2 diabetes (10 each in 4 groups: placebo/no medication, Emulin/no medication, placebo/metformin and Emulin/metformin) were evaluated. At the end of 1 week, fasting blood glucose, 2 h postprandial, actual peak glucose, and AUC (post-50 g OGTT) were determined. The placebo-only group had a large (5%–13%) increase in all parameters. The Emulin group and those on metformin performed similarly with reductions between 1% and 5%, with Emulin slightly outperforming the medication-alone group. The most significant reduction occurred in the Emulin/metformin group, with decreases in the parameters by up to 20%. These results suggest that Emulin, if consumed regularly, could not only have the acute effect of lowering the glycemic impact of foods, but chronically lower background blood glucose levels of type 2 diabetics.

KEY WORDS: ● chlorogenic acid ● flavonoids ● flavonols ● myricetin ● polyphenols ● queretin

INTRODUCTION

TYPE 2 DIABETES IS CONSIDERED an epidemic worldwide in industrialized nations.1,2 Historically, the incidence of type 2 diabetes has tracked the increased consumption of refined carbohydrates.3-6 Type 2 diabetic patients have a combination of deficits, including insulin resistance and beta cell failure leading to fasting and postprandial hyperglycemia. The body has a tremendous capacity to maintain homeostasis. Therefore, DeFronzo7 points out that several key metabolic pathways associated with diabetes, such as gluconeogenesis and glucose transport, must be addressed simultaneously in order for the body to make headway against the disease and be able to reset at a lower blood glucose level. In considering DeFronzo’s proposal, an effective compound to ameliorate the symptoms of diabetes, or prevent its onset, might:

- Inhibit conversion of complex carbohydrates to simpler sugars
- Inhibit the absorption of glucose from the gut into the bloodstream
- Enhance the absorption of glucose from the bloodstream and its utilization by muscle cell mitochondria
- Inhibit new fat formation
- Inhibit gluconeogenesis
- Increase insulin sensitivity and production

Traditional treatment for diabetes has included alterations in diet to avoid refined carbohydrates and prescribing of drugs to inhibit hyperglycemia and increase insulin release. Common drugs for the treatment of diabetes or its symptoms are listed in Table 1. All are synthetic, target at most two metabolic pathways, and have moderate or severe side effects. Natural compounds with excellent safety profiles have been shown to ameliorate the symptoms of diabetes by affecting various pathways and have been studied for decades.8

Inhibition of glucosidase

Tarlo et al.5 screened 30,000 crude biological extracts of terrestrial and marine origin for competitive inhibition of amylase, the most potent of which were found to have a myricetin core. Additionally, significant alpha glucosidase activity is inhibited by chlorogenic acid in humans,10,11 by myricetin and queretin in swine,12,13 and in rat in vitro by queretin.13 This may be the underlying metabolic effect where chlorogenic acid-enriched coffee with glucose causes weight loss in humans.14 Similarly, 0.5% Gross Coffee Bean Extract, of which chlorogenic acid is a major constituent, has been shown to reduce visceral fat content and body weight in mice.15

Effects on glucose transport

Robust noncompetitive inhibition of glucose and fructose transport by glucose transporter 2 (GLUT2) is produced by the flavonols myricetin and queretin.16 Intravenous administration (1 mg/kg) of myricetin causes a decrease in the plasma glucose concentration and insulin resistance of...
Table 1. Current Drug Classes Prescribed for the Treatment of Type 2 Diabetes

<table>
<thead>
<tr>
<th>Class</th>
<th>Action</th>
<th>Side Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sulfonylureas</td>
<td>Secretagogues—upregulate the secretion of insulin</td>
<td>Weight gain, Hypoglycemia</td>
</tr>
<tr>
<td>Meglitinides</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biguanides (metformin)</td>
<td>Down-regulate gluconeogenesis</td>
<td>Diarrhea</td>
</tr>
<tr>
<td></td>
<td>Uregulate glucose transporter 4</td>
<td>Nausea</td>
</tr>
<tr>
<td>Thiazolidinediones</td>
<td>Uregulate mRNA transcription leading to decreased insulin resistance</td>
<td>Osteoporosis (in females), fluid retention, weight gain, Flarelence</td>
</tr>
<tr>
<td>Alpha-glucosidase inhibitors</td>
<td>Downregulate starch digestion</td>
<td>Nausea, weight loss, pancreatitis</td>
</tr>
<tr>
<td>GLP analogs</td>
<td></td>
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</tbody>
</table>

Obese Zucker rats by increasing the expression of glucose transporter 4 (GLUT4) and increasing the protein levels and phosphorylation of insulin receptor substrate-1 in soleus muscle of these obese rats. At the same time, myricetin has been shown to inhibit the same transporter GLUT4 in vitro in normal rat adipocytes. This different activity in different tissues, or tissues of the same type in different organs, may be due to a difference in membrane fluidity.

Inhibition of adipocyte growth and development

Myricetin coupled with quercetin together work to inhibit net glucose from entering fat cells and release glucose from fat tissue in rat adipocytes. Preadipocyte growth or development into adipocytes is inhibited in vitro by quercetin and chlorogenic acid.

Inhibition of gluconeogenesis

Quercetin inhibits gluconeogenesis in rat liver slices, which appears to be by inhibition of glucokinase and glucose 6-phosphatase.

In a study of streptozotocin-treated rats, equal amounts of myricetin, chlorogenic acid, and quercetin (total 15 mg/kg) were administered three times per day via gavage; at the end of 1 week, midday background blood glucose and postprandial area under the curve (AUC) blood glucose dropped by 18% and 27%, respectively (Koetzner L: Personal communication. Eurofins Product Safety Laboratories, Dayton, NJ, USA).

A main reason for noncompliance (patients not taking their medication) associated with diabetic pharmaceuticals is gastro-intestinal issues. Capsules containing 500 mg of guava leaf extract normalized for quercetin content administered every 8 h during 3 days is effective in reducing abdominal pain associated with acute diarrhea in humans.

Oral treatment with quercetin, which appears to be converted to quercetin in the small intestine, reduces myeloperoxidase and alkaline phosphatase levels, preserves normal fluid absorption, counteracts glutathione depletion, and ameliorates colonic damage. Quercetin-treated rats have less diarrheal output and do not show mucosal hyperplasia.

It would appear from these previous studies that a formulation of flavonoids containing myricetin, chlorogenic acid, and quercetin would work in combination on several pathways to ameliorate the symptoms of diabetes and address much of DeFronzo’s concerns, while at the same time alleviate gastrointestinal distress. The purpose of this study is to evaluate whether Emulin (Anderson Global Group LLC, Irvine, CA, USA), a recently available proprietary patented formulation of myricetin, quercetin, and chlorogenic acid, the combination and ratio of which do not themselves occur in nature, is able to reduce fasting and postprandial plasma blood glucose in human type 2 diabetics, while avoiding gastrointestinal upset.

MATERIALS AND METHODS

This was a randomized, double-blind, placebo controlled, parallel group study. Test article identity was unknown to principle investigator, clinic staff, or subjects. This research was fully reviewed by Quorum Review IRB (Seattle, WA, USA) and procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008. Forty subjects were selected based upon the following criteria:

Inclusion criteria:

- Male or female age 18 or older
- Females of childbearing potential must agree to use a medically approved method of birth control and have a negative urine pregnancy test result
- Diagnosed with type 2 diabetes (having a blood sugar level between 126–249 mg/dL)
- Body mass index (BMI) ≥ 30 kg/m²

Exclusion criteria:

- Women who are pregnant, breastfeeding, or planning to become pregnant during the course of the trial
- Unstable medical conditions as determined by the Principle Investigator
- Use of insulin
- Participation in a clinical research trial within 30 days before randomization
- Subjects with type 1 diabetes

Subjects were screened to meet the inclusion and exclusion criteria. An insulin C-peptide blood test was performed to show that individuals did not have type 1 diabetes. At screening (visit 1, week 0), a urine pregnancy test was conducted on all females of childbearing potential. Medical history and concomitant therapies were reviewed; height, weight, heart rate, and blood pressure were measured and BMI calculated. Fasting blood samples were collected for determination of glucose from eligible subjects. Subjects were divided into two groups: those on metformin, and those on no drug regime. As subjects of both groups were enrolled they were assigned into group A or group B without prejudice. This
resulted in four groups: (1) Placebo only, (2) Emulín only, (3) Placebo and on metformin, and (4) Emulín and on metformin. Subjects were administered one 250 mg capsule (placebo or Emulín, according to group assignment) 15 min before undergoing oral glucose tolerance test (OGTT) with blood sampling for glucose at 30, 60, 120, 180, and 240 min after ingesting a 50 g glucose tolerance beverage (Ensure) within 5 min. Bottles (containing placebo or Emulín capsules, marked “A” or “B”) and a treatment diary were dispensed and subjects instructed on use. Subject self-administered one 250 mg capsule 15 min before breakfast, lunch, and dinner every day except on day of next OGTT, where the dose at breakfast was skipped. Subjects were instructed to record food intake, remain on their typical diet, and to fast 12 h before returning to the clinic for the next OGTT.

Subjects returned to the clinic for visit 2 on day 7 (week 1). Weight, heart rate, and blood pressure were measured and OGTT performed as on visit 1. Diaries were collected; compliance, concomitant therapies, and adverse events were assessed and addressed.

Blood glucose for each individual in Group A and Group B were determined for points at 0, 30, 60, 120, 180, and 240 min for each visit (two visits). The time zero for the OGTT served as the fasting blood glucose data point. AUCs were determined for each individual for each visit using the trapezoid method. Percent changes in fasting, peak postprandial, 2 h postprandial, and AUC glucose were determined for each individual within each treatment group.

P-values were determined with a Mann–Whitney U-test for all combinations of pairs.

RESULTS AND DISCUSSION

Fasting blood glucose levels >125 mg/dL and 2 h postprandial glucose >199 mg/dL are indicative of diabetes. The body’s carbohydrate metabolism fails to adequately control blood sugar. Reducing these two major indicators could ameliorate the effects of diabetes. Table 2 shows the average percent changes in fasting, 2 h postprandial, actual peak, and AUC blood glucose at 1 week for all treatment groups.

Fasting blood glucose

There was a large increase in the placebo group (12.75%), while there was a decrease in the Emulín and metformin groups at α=0.01 and 0.10, respectively. Emulín outperformed the metformin group, which remained unchanged, but the difference between the groups was not statistically significant. Those persons on metformin receiving Emulín showed over 30-fold increase in efficacy, significant at α=0.05, the effect more than additive, indicating the test article is impacting other pathways associated with fasting blood glucose than those affected by metformin alone. It appears the body has reset its fasting blood glucose at a lower level in response to Emulín or the combination of Emulín and metformin. Such a dramatic increase in the placebo group can

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**Table 2. Statistical Significance Between Groups at the Indicated Alpha Level**

<table>
<thead>
<tr>
<th>Group</th>
<th>% Change</th>
<th>α=0.20</th>
<th>α=0.10</th>
<th>α=0.05</th>
<th>α=0.01</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting blood glucose</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo</td>
<td>12.75</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td>Emulín</td>
<td>5.11</td>
<td>B</td>
<td>B</td>
<td>D</td>
<td>B</td>
</tr>
<tr>
<td>Metformin</td>
<td>0.59</td>
<td>B</td>
<td>C</td>
<td>B</td>
<td>C</td>
</tr>
<tr>
<td>E+M</td>
<td>-19.53</td>
<td>D</td>
<td>D</td>
<td>C</td>
<td>D</td>
</tr>
<tr>
<td>2 h postprandial</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo</td>
<td>-4.94</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td>Emulín</td>
<td>-1.01</td>
<td>A</td>
<td>B</td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>Metformin</td>
<td>-3.50</td>
<td>B</td>
<td>C</td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>E+M</td>
<td>-13.42</td>
<td>D</td>
<td>C</td>
<td>D</td>
<td>B</td>
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<tr>
<td>Actual peak</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo</td>
<td>9.91</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
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<tr>
<td>Emulín</td>
<td>1.15</td>
<td>A</td>
<td>B</td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>Metformin</td>
<td>-0.81</td>
<td>B</td>
<td>C</td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>E+M</td>
<td>-5.14</td>
<td>B</td>
<td>C</td>
<td>D</td>
<td>B</td>
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<tr>
<td>Area under the curve</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo</td>
<td>11.50</td>
<td>A</td>
<td>A</td>
<td>A</td>
<td>A</td>
</tr>
<tr>
<td>Emulín</td>
<td>-5.06</td>
<td>B</td>
<td>B</td>
<td>B</td>
<td>A</td>
</tr>
<tr>
<td>Metformin</td>
<td>-2.95</td>
<td>B</td>
<td>C</td>
<td>B</td>
<td>C</td>
</tr>
<tr>
<td>E+M</td>
<td>-16.88</td>
<td>B</td>
<td>C</td>
<td>D</td>
<td>B</td>
</tr>
</tbody>
</table>

*Groups are as follows: placebo, taking a placebo and not on any drug regime; Emulín, taking Emulín and not on any drug regime; metformin, on metformin and taking a placebo; E+M, taking Emulín and on metformin.

*Percent change is the average percent change in the parameters between day 0 and day 7 for all individuals in the group.

*Values followed by the same letter are not significantly different at the indicated alpha level.
be explained by the patients taking the liberty to change their diets to include more glucose-rich foods.

**Two hour postprandial and peak glucose**

As with the fasting blood glucose, for the 2 h postprandial blood glucose there was an increase in the placebo group; however, smaller at 4.9%. There was no significant difference between the placebo, Emulin, and metformin only groups at \( \alpha = 0.10 \). However, the combination of Emulin and metformin group (E + M) decreased 13% and was significantly different contrasted to the placebo group at \( \alpha = 0.05 \). The actual peak glucose showed a very similar pattern except the increase in the placebo group and the decrease in the E + M group was about double and one third that of the 2 h postprandial changes, respectively.

**Area under the curve**

Fasting blood glucose and AUC showed a remarkably similar pattern and levels of significance. Although fasting blood glucose and peak glucose levels are indicators for diabetes, AUC is most often used to determine the effectiveness of treatments for the disease. With no treatment, the placebo group had an increase in this parameter of 11.5% by the end of the week, while the Emulin alone group experienced a 5% decrease. Metformin alone was slightly less effective at reducing AUC (3%) than Emulin alone. When taken with Emulin, the reduction was >16%. While the effects appear to be more than additive, there was no significant difference between the Emulin and metformin groups. The data presented in Table 2 is visually represented in Figures 1 and 2. The acute effect of Emulin (the effect which occurs immediately or within hours of administr-
blood glucose levels of type 2 diabetics. Further research is warranted.

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AUTHOR DISCLOSURE STATEMENT

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REFERENCES


