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Beneficial metabolic effects of dietary epigallocatechin gallate alone and in combination with exendin-4 in high fat diabetic mice

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ABSTRACT

Objective: Significant attempts are being made to generate multifunctional, hybrid or peptide combinations as novel therapeutic strategies for type 2 diabetes, however this presents key challenges including design and pharmaceutical development. In this study, we evaluated metabolic properties of oral nutritional supplement epigallocatechin gallate (EGCG) in combination with GLP-1 agonist exendin-4 in a mouse model of dietary-induced diabetes and obesity.

Methods: EGCG, exendin-4 or combination of both were administered twice-daily over 28 days to high fat (HF) mice on background of low-dose streptozotocin. Energy intake, body weight, fat mass, glucose tolerance, insulin sensitivity, lipid profile, biochemical and hormone markers, and islet histology were examined.

Results: All treatment groups exhibited significantly reduced body weight, fat mass, circulating glucose and insulin concentrations, and HbA1c levels which were independent of changes in energy intake. Similarly, there was marked improvement in glycaemic control, glucose-stimulated insulin release, insulin sensitivity, total cholesterol and triglycerides, with most prominent effects observed following combination therapy. Circulating corticosterone concentrations and 11beta-hydroxysteroid dehydrogenase type1 (11β-HSD1) staining (in pancreas) were beneficially decreased without changes in circulating interleukin 6 (IL-6), alanine transaminase (ALT) and glutathione reductase. Combination therapy resulted in increased islet area and number, beta cell area, and pancreatic insulin content. Generally, metabolic effects were much more pronounced in mice which received combination therapy.

Conclusions: EGCG alone and particularly in combination with exendin-4 exerts positive metabolic properties in HF mice. EGCG may be useful dietary adjunct alongside GLP-1 mimetics in treatment of diabetes and related disorders.

1. Introduction

Glucagon-like peptide-1 (GLP-1) is an amidated 30-amino acid gut peptide secreted from enteroendocrine L-cells in response to nutrient ingestion (Reimann et al., 2016). Upon secretion, GLP-1 binds to the GLP-1 receptor which is highly expressed in pancreatic beta cells and other extra-pancreatic tissues including the brain, heart, kidney, lung, enteric and peripheral nervous systems (Yamada et al., 2016). The most widely recognised and characterised physiological action of GLP-1 is its ability to stimulate glucose-induced insulin secretion (Tudurí et al., 2016). However, GLP-1 also inhibits glucagon release, delays gastric emptying, lowers body weight and induces satiety (Tahrani et al., 2016). Furthermore, research in animal models of diabetes-obesity and neurodegenerative disease show that GLP-1 improves cognition, synaptic plasticity and enhances hippocampal neurogenesis, further highlighting GLP-1 as an extremely attractive therapeutic agent (Gault et al., 2010; Gengler et al., 2012; Porter et al., 2010; Cai et al., 2016). Yet, like numerous gut peptides, native GLP-1 is quickly degraded in the circulation by the enzyme dipeptidylpeptidase-4 (DPP4) and, to circumvent this, DPP4 inhibitors and stable GLP-1 receptor agonists have been developed. The first approved GLP-1 receptor agonist, exenatide (Byetta®), was isolated (as exendin-4) from the salivary gland of the Gila monster (*Heloderma suspectum*) (Eng et al., 1992). Exendin-4 is a 39-amino acid peptide which has 53% sequence homology with human GLP-1, and contains

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glycine at position 2 instead of alanine, and an additional C-terminal amino acid sequence of PSSGAPPPS (Raufman, 1996). These modifications protect exendin-4 against DPP4 degradation and promote formation of secondary structure, leading to enhanced pharmacokinetic properties, longer circulating half-life and extended duration of action (Lovshin et al, 2009).

Even though a range of GLP-1 receptor agonists are now available, it has become increasingly evident that successful management of patients with type 2 diabetes requires the further development of safe and more effective mono and/or combination therapies with complementary mechanisms of action. At present, considerable effort is being made to generate multifunctional, hybrid or peptide combinations as novel therapeutic strategies (Henderson et al., 2016; Trevaskis et al., 2015; Dalbøge et al., 2014; Finan et al., 2015; Clemmensen et al., 2014; Irwin et al., 2015; Gault et al., 2013; O'Harte et al., 2016; Bhat et al., 2013). However, this approach poses several key challenges especially with regards to designing an appropriate and balanced pharmaceutical entity and moreover, likely requirements for the patient to increase the frequency and/or number of injections. One alternative possibility is to combine the powerful effects of a GLP-1 receptor agonist such as exenatide with a widely available oral nutritional supplement such as epigallocatechin gallate (EGCG) which is an antioxidant polyphenol found in green tea (Suzuki et al., 2016).

EGCG is an ester of epigallocatechin and gallic acid and is the most abundant catechin found in green tea. It has been shown to offer a range of beneficial effects against cancer, obesity, atherosclerosis and infection (Suzuki et al., 2016; Niedzwiecki et al., 2016; Chowdhury et al., 2016). There is also evidence to link EGCG with body weight loss, release of endocrine pancreatic hormones, appetite suppression, improvement in glucose tolerance, augmentation of glucose-stimulated insulin secretion and enhanced insulin sensitivity (Nishiumi et al., 2010; Yang et al., 2016; Kao et al., 2000; Wolfram et al., 2006; Ortsäter et al., 2012). While the precise underlying cellular, biochemical and molecular mechanisms by which EGCG ameliorates metabolic disease are not fully understood, emerging data point to EGCG as an inhibitor of the microsomal enzyme 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) which decreases local cortisol concentrations and alleviates insulin resistance (Hintzpeter et al., 2014). Indeed, several 11β-HSD1 inhibitors have been developed and data suggests that they improve glycaemic control, lipid profile and blood pressure, with moderate body weight loss (Anderson et al., 2013). Thus, we hypothesised that combination of exendin-4 with the oral dietary supplement EGCG would provide improved metabolic outcomes. High fat diabetic mice were treated with EGCG alone, exendin-4 alone and a combination of both over a 28-day period. Effects on body weight, glycaemic control, insulin secretion and action, lipids, circulating biomarkers, and islet histology were assessed.

2. Materials and methods

2.1. Chemicals

Exendin-4 (molecular mass: 4186.6 Da) was purchased from GL Biochem Ltd. (Shanghai, China). (-)-Epigallocatechin gallate purified from green tea (EGCG; molecular weight 458.37 g/mol) and streptozotocin were purchased from Sigma-Aldrich (Poole, Dorset, UK). All other chemicals were obtained from standard sources and of the highest purity available.

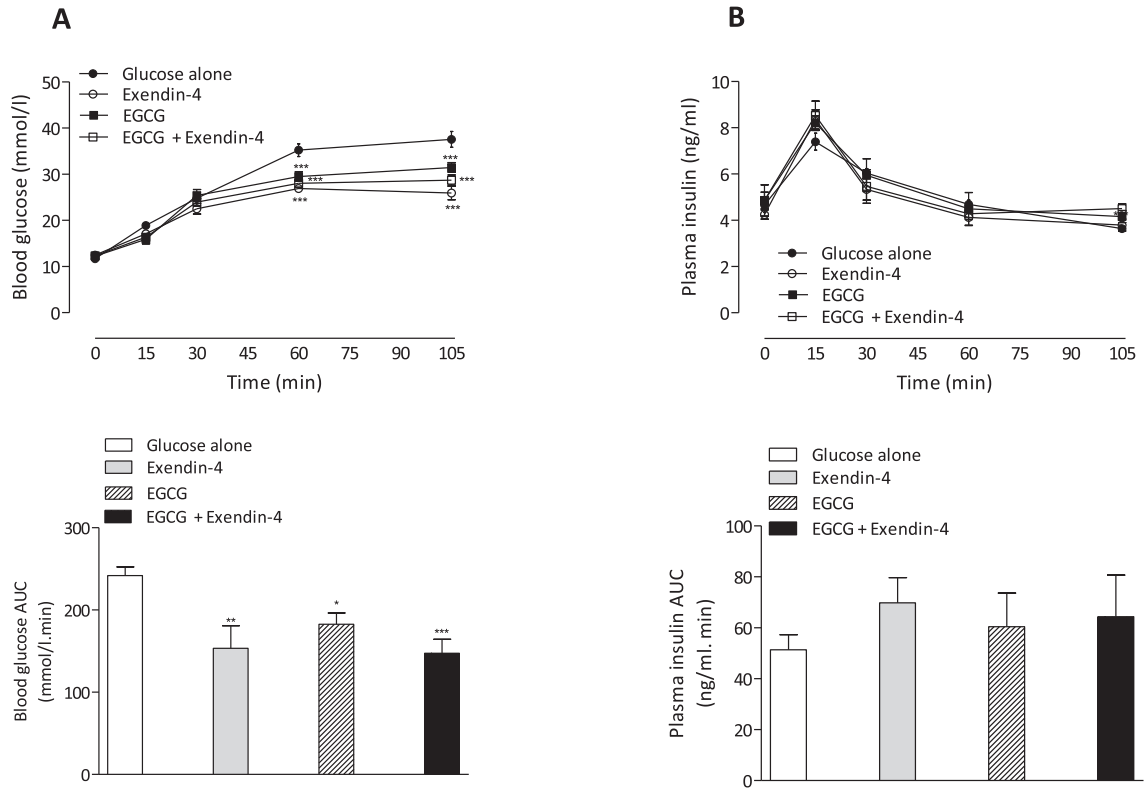

Fig. 1. Acute effects of EGCG administered alone and in combination with exendin-4 on glucose and insulin concentrations in *db/db* mice. (A) Glucose and (B) insulin concentrations were measured prior to and after administration of glucose alone (18 mmol/kg; i. p.) or in combination with EGCG (50 mg/kg; p. o.), exendin-4 (25 nmol/kg; i. p.), or a combination of the two. Glucose and insulin AUC values for 0–105 min post-injection are also shown. Values are mean ± SEM (n = 6 mice). *P < 0.05, **P < 0.01 and ***P < 0.001 compared to glucose alone.

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