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RESEARCH PAPER

Effect of Lipid Excipients on In Vitro Pancreatic Lipase Activity

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ABSTRACT

Purpose. To study the effects of two lipid excipients, Peceol® and Gelucire 44/14® on the in vitro pancreatic lipase activity. *Methods*. A 50 μL reaction mixture, consisting of 45 μL (^{3}H) triolein as the radiolabeled substrate, 2.5 μL Peceol or Gelucire 44/14 (0.05–0.5%), either alone or in combination, 2.5 μL colipase (100 μg/mL), and 2.5 μL pancreatic lipase (1 mg/mL), was incubated for 10 min at room temperature. At the end of incubation, the reaction was stopped by the addition of an extraction solvent containing chloroform, methanol, and *n*-heptane (12.5:14:10), and the mixture vortexed briefly. Subsequently, 250 μL of 50 mM sodium carbonate was added and the aqueous and organic phase separated by centrifugation for 5 min at 1000 g. One hundred microliters of the supernatant was transferred to a scintillation counter and then radioactivity measured after the addition of 3.6 mL of scintillation fluid. Pancreatic lipase activity was determined by measuring the amount of free fatty acid released into the incubation medium and expressed as μmol free fatty acid released/min. *Results*. When used alone, Peceol inhibited the pancreatic lipase activity significantly in a concentration-dependent manner, with a maximum inhibition of 57% at 0.4% of the excipient [$p < 0.05$, one-way analysis of variance (ANOVA)]. Similarly, Gelucire 44/14 alone caused inhibition of lipase activity in a concentration-dependent manner. However, the maximum inhibition (30%) was smaller in magnitude compared with the former agent. When the two excipients were used in combination, the inhibitory effects on the enzyme activity were similar to those observed with the individual agents ($p < 0.05$, one-way ANOVA). However, the maximum inhibition of 30% was lower than that observed with Peceol alone. *Conclusions*. The results from this study suggest that these lipid excipients inhibit in vitro pancreatic lipase activity and should be taken into consideration when developing oral formulations using these agents.

Key Words: Peceol; Gelucire 44/14; Pancreatic lipase activity; Oral formulations.

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INTRODUCTION

Over 65% of commercially available drugs are formulated for oral administration.[1–3] However, one of the major factors limiting the effectiveness of orally administered drugs is poor absorption from the gastrointestinal tract or extensive presystemic clearance through hepatic first-pass metabolism.[1–3] For poorly water-soluble drugs, slow dissolution rate in the primarily aqueous contents of the gastrointestinal tract presents a significant barrier to absorption.[1–5] One strategy for improving the absorption of these drugs involves administration in a lipid-based delivery system,[6–11] which presents the drug to the gastrointestinal tract in a solubilized form, thus eliminating poor aqueous solubility, and slows dissolution rate as barriers to absorption.

Lipid excipients are routinely used in pharmaceutical formulations, either as vehicles or solubilizers of drugs that are otherwise insoluble or poorly soluble in an aqueous medium. Two such lipid excipients are Peceol® and Gelucire 44/14®. Gelucire 44/14 is an agent that forms a very fine emulsion when in contact with the gastrointestinal fluids in vivo.[4] It consists of a mixture of mono-, di-, and triglycerides, accounting for approximately 19% of the total lipid mass, with the remaining 81% being comprised primarily of polyethyleneglycol (PEG) 1500 mono- and diesters of C_8–C_{18} fatty acids (73%) and a small amount of free PEG 1500 (8%).[4] Peceol is a readily dispersible, lymphotropic solubilizing agent comprised primarily of a mixture of mono- and diglycerides of oleic acid, which closely resembles the end-products of intestinal lipid digestion.[4–6] Peceol and Gelucire 44/14 have been used in self-emulsifying drug delivery systems because of the ability of this combination to solubilize water-insoluble drugs in high concentration while providing a semisolid delivery system with rapid self-emulsifying properties and supporting lymphatic drug transport.[6]

However, it is unknown if these agents affect enzymes of the gastrointestinal tract responsible for the metabolism and subsequent absorption of dietary lipids and fats. One such critical enzyme is pancreatic lipase, an enzyme responsible for the hydrolysis of triacylglycerol into monoacyglycerol and nonesterified fatty acids.[12] The following studies have been designed to investigate if Peceol and Gelucire 44/14, alone and in combination, would affect pancreatic lipase/colipase-mediated in vitro triacylglycerol metabolism. Based on the composition of these excipients, we hypothesized that they would inhibit pancreatic lipase activity.

MATERIALS AND METHODS

Materials

The radiolabeled substrate (^{3}H) triolein was purchased from Amersham Life Sciences (Mississauga, Ontario, Canada). Unlabeled triolein, taurodeoxycholic acid, sodium carbonate, porcine pancreatic lipase, and colipase from porcine pancreas were obtained from Sigma Inc. (Mississauga, Ontario, Canada). All other chemicals as well as solvents for extraction were purchased from Sigma Inc. Peceol and Gelucire 44/14 were donated as a gift from Gatte Fosse Canada (Montreal, Canada).

Methods

Preparation of Reagents

Prior to the experiment, stock solutions containing 1 mg/mL pancreatic lipase and colipase were prepared in a buffer containing 10 mM Tris HCl (pH 8.0) and 0.15 M NaCl. Aliquots of pancreatic lipase solution were stored at −80°C, whereas aliquots of colipase solution were stored at −20°C. In addition to the lipases, an assay buffer containing 30 mM Tris HCl (pH 8.0), 1 mM $CaCl_2$, and 4 mM taurodeoxycholic acid was also prepared.[12]

On the day of the experiment, 2 μL triolein was added to 5 mL of the assay buffer to give a final triolein concentration of 0.312 mM. The radiolabeled substrate solution was prepared by mixing 3 μL (^{3}H) triolein with each mL of the assay buffer and sonicating for 5 mL in a bath sonicator. The colipase stock solution was diluted 1:10 with the assay buffer prior to using in the experiment.

The extraction solvent was prepared by mixing chloroform, methanol, and heptane in the ratio of 12.5:14:10 (v/v/v).[12]

Pancreatic Lipase Assay Procedure

On the day of the experiment, 45 μL of the radiolabeled substrate solution was mixed with 2.5 μL of either Peceol (0.05–0.5% v/v) or Gelucire (0.05–0.5% v/v) or an equal mixture of Peceol and Gelucire 44/14 (0.05–0.5% v/v), 2.5 μL colipase (1:10), and 2.5 μL pancreatic lipase to give a total reaction volume of 50 μL.[12] The reaction mixture was incubated at room temperature for 10 min and the reaction stopped by adding 0.75 mL of the

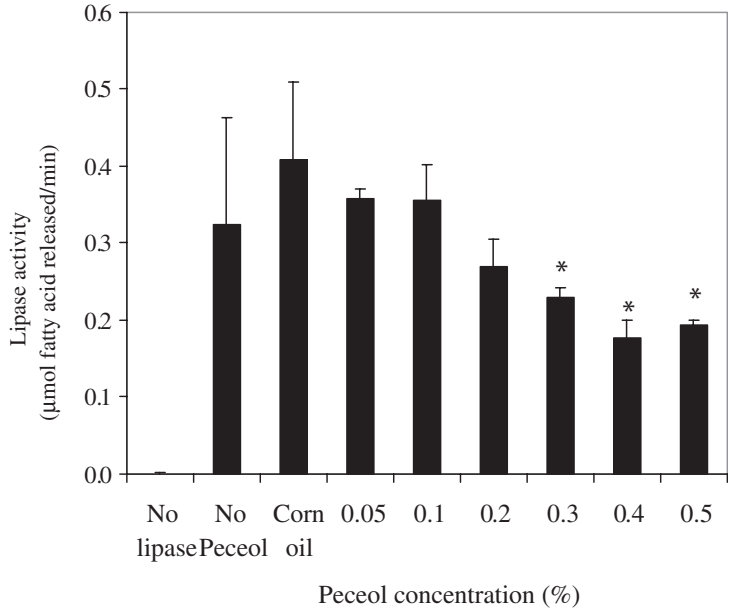

Figure 1. Effect of Peceol (0.05–0.5% v/v) alone on pancreatic lipase activity. *Key:* *Significantly different from corn oil alone; $p < 0.05$, one-way ANOVAfollowed by Bonferroni post-hoc test. Data presented as mean ± standard error of the mean (S.E.M.); $n = 6$.

extraction solvent. The samples were mixed briefly by vortexing, followed by the addition of 0.25 mL of 50 mM sodium carbonate. The mixture was vortexed vigorously and the aqueous and organic phases were separated by centrifugation for 5 min at 1000 g. Finally, 0.1 mL of the supernatant aqueous phase was transferred to a scintillation vial, 3.6 mL of scintillation fluid was added, and the radioactivity was measured in a liquid scintillation counter. The pancreatic lipase activity was measured as the amount of free fatty acid released and expressed as μmol free fatty acid released/min.[12]

Statistical Analysis

Each data point represents the average of six replicates. Data is expressed as mean ± standard error of the mean (S.E.M). The data was analyzed by one-way analysis of variance (ANOVA), followed by a Bonferroni post-hoc test. Data was considered to be significantly different if $p < 0.05$.

RESULTS AND DISCUSSION

The observations from this study demonstrate that, when used alone, Peceol produces significant concentration-dependent inhibition of pancreatic lipase activity, with maximum inhibition of 57% occurring at 0.4% (v/v) (Fig. 1). Gelucire alone produced a similar concentration-dependent inhibition of the enzyme activity. However, the maximal inhibition was observed at 0.2% (v/v) (Fig. 2). When compared to Peceol, the magnitude of inhibition produced by Gelucire was smaller with maximum inhibition of ~30%.

Experiments were also performed to determine whether the inhibition of pancreatic lipase activity by 0.4% (v/v) Peceol was influenced by the length of incubation. At 15, 30, and 45 min of incubation, the enzyme activity was inhibited by 6%, 38%, and 54%, respectively (Fig. 3). However, in this experiment, the control pancreatic lipase activity in the presence of corn oil alone was also decreased with increasing length of incubation.

When Peceol and Gelucire were used in combination at a concentration range of 0.05–0.5% (v/v), the lipase activity was inhibited in a concentration-dependent manner, with maximum inhibition of 30% at 0.2% (v/v) of the mixture, which was statistically significant (Fig. 4).

Young and Hui have recently reported that partial hydrolysis of the triacylglycerol into monoacyglycerol and nonesterified fatty acids by pancreatic lipase/colipase is required for dietary cholesterol

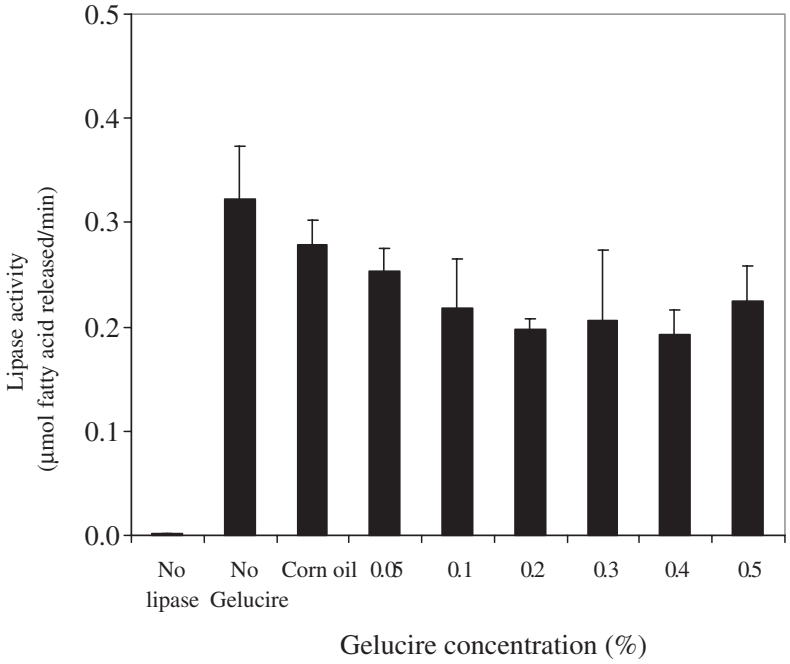

Figure 2. Effect of Gelucire (0.05–0.5% v/v) alone on pancreatic lipase activity. *Key*: *Significantly different from no Gelucire; $p < 0.05$, one-way ANOVA followed by Bonferron post-hoc test. Data presented as mean ± standard error of the mean (S.E.M.); $n = 6$.

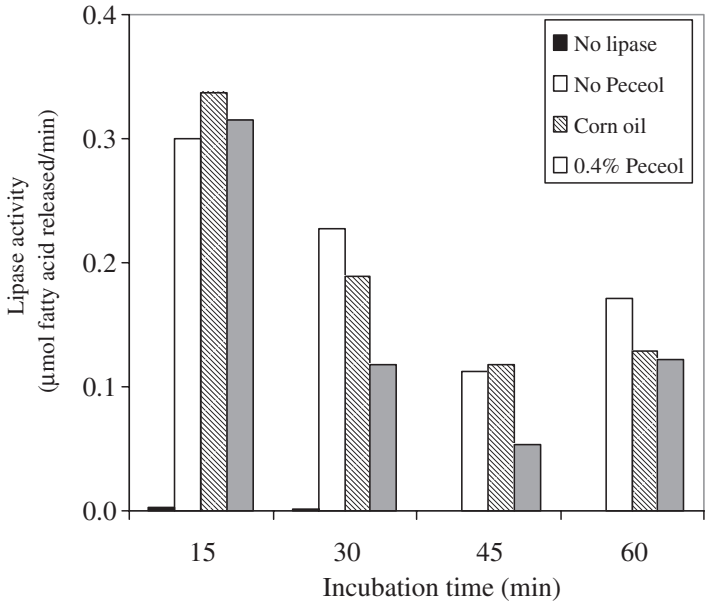

Figure 3. Effect of incubation time on pancreatic lipase activity in the presence of 0.4% v/v Peceol. *Key:* *Significantly different from no Peceol; $p < 0.05$, one-way ANOVA followed by Bonferroni post-hoc test. Data presented as mean ± standard error of the mean (S.E.M.); $n = 6$.

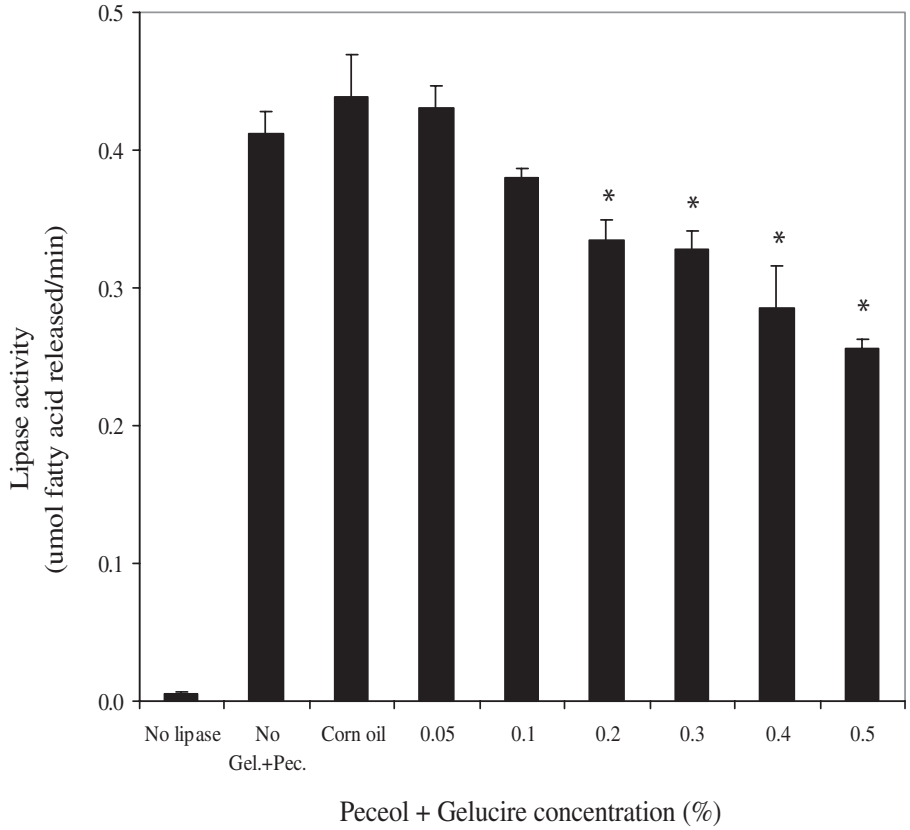

Figure 4. Effect of Peceol and Gelucire (0.05–0.5% v/v), in combination on pancreatic lipase activity. *Key:* *Significantly different from corn oil alone; $p < 0.05$, one-way ANOVA followed by Bonferroni post-hoc test. Data presented as mean ± standard error of the mean (S.E.M.); $n = 6$.

that is transported as emulsified substrates to be absorbed by intestinal cells.[12] Thus, the inhibition of triacylglycerol digestion may impact on the absorption efficiency of dietary cholesterol in the intestinal lumen. Furthermore, these agents may alter the activity of other key gastrointestinal tract enzymes,[13] and studies to investigate these enzymes are warranted.

In conclusion, the results from this study suggest that these lipid excipients inhibit in vitro pancreatic lipase activity and should be taken into consideration when developing oral formulations using these agents.

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