JPP Journal of Pharmacy and Pharmacology

JPP 2006, 58: 1099–1105

Received February 2, 2006
Accepted April 13, 2006
DOI 10.1211/jpp.58.8.0011
ISSN 0022-3573

Inhibition of pancreatic lipase by poloxamer 407 may provide an adjunct treatment strategy for weight loss

Thomas P. Johnston and Ira J. Goldberg

Abstract

This investigation was conducted to evaluate whether poloxamer 407 (P-407), a nonionic surface-active-agent that functions as a lipase inhibitor, could aid in weight loss by inactivating pancreatic lipase (PL) following oral administration to mice. Using a pH-titrimetric method, P-407 was evaluated for its ability to inhibit PL activity in-vitro. The palatability of drinking water containing P-407 (50 μM) was assessed in mice to determine whether inclusion of P-407 altered either the volume of water ingested, or the volume of urine produced, per day. P-407 at the same concentration was next evaluated for its potential to mediate weight loss over a one-month period in mice fed a high-fat diet. Faecal fat determinations and the potential for P-407 to lower plasma triacylglycerol concentrations following oral administration of a standard lipid emulsion were also conducted. P-407 was determined to have an IC50 of 15.9 μM in-vitro. Inclusion of P-407 in drinking water neither perturbed the daily volume of water ingested, nor the volume of urine produced. Over the course of one month, adult mice, which were fed the high-fat diet and treated with P-407, lost approximately 12.4 ± 1.7% of their initial body weight, whereas, control mice fed the identical diet continued to slowly gain weight (7.3 ± 0.5% of their initial body weight). The amount of total lipids excreted in the faeces of high-fat-fed, P-407-treated mice was approximately 45% greater than that observed for control mice eating the same diet. Lastly, plasma triacylglycerol concentrations following oral administration of the standard lipid emulsion containing P-407 were significantly lower than corresponding plasma triacylglycerol concentrations observed in mice administered the lipid emulsion alone. While not as potent as orlistat, P-407 may potentially represent an additional treatment strategy for weight loss, especially when combined with caloric restriction, regular exercise, and anti-obesity medications of other drug classes.

Introduction

Obesity is among the most common and serious health problems in the US. The Centers for Disease Control estimates that approximately two-thirds of the US population, both adults and children, are overweight, which translates to approximately 200 million people (Lethbridge-Cejku et al 2004). Excess body weight is directly associated with a higher mortality rate (Bray 1985). Moreover, being overweight and obese is associated with additional risk factors for increased mortality, specifically hypertension (Pi-Sunyer 1993), hyperlipidaemia (Van Itallie 1985; Pi-Sunyer 1993), and diabetes mellitus (US National Commission on Diabetes 1975), as well as other chronic diseases, such as cancer (endometrial, cervical, ovarian, breast, prostate, gallbladder, and colon (Garfinkel 1985)), restrictive lung disease, sleep apnoea, gout, osteoarthritis, and thromboembolic disease, to name but a few (Pi-Sunyer 1993).

Unfortunately, the treatment of obesity is difficult. Of the many options for treating obesity, behaviour modification, including dietary changes, is preferred (NIH Consensus Statement 1991; Bray 1993). Although behaviour incorporating dietary restrictions and appropriate exercise is the preferred treatment for obesity, pharmacologic therapy of obesity is an area of continued research and development. Essentially, the pharmacological agents used for the treatment of obesity fall into four major categories. Firstly, there are appetite suppressants (e.g. centrally-acting adrenergic agents (benzphetamine etc.), serotonergic agents (fenfluramine – currently withdrawn

Division of Pharmaceutical Science, School of Pharmacy, University of Missouri-Kansas City, Kansas City, MO, USA

T. P. Johnston

Department of Preventative Medicine and Nutrition, Columbia University College of Physicians and Surgeons, New York, NY, USA

I. J. Goldberg

Correspondence: T. P. Johnston, Division of Pharmaceutical Science, Rm. 211A, School of Pharmacy, University of Missouri-Kansas City, 5005 Rockhill Road, Kansas City, Missouri 64110-2499, USA. E-mail: johnstont@umkc.edu

from the US market, etc.), and adrenergic/serotonergic agents (sibutramine)). Secondly, there are thermogenic agents (e.g. adrenergic agents (ephedrine-caffeine) and β_3-agonists (BRL 26830A etc.)). Thirdly, there are digestion inhibitors (e.g. lipase inhibitors (orlistat), carbohydrate-based fat substitutes, protein-based fat substitutes, and fat-based fat substitutes (olestra)). Fourthly, there is hormonal manipulation (e.g. leptin analogues and neuropeptide Y antagonists) (Cerulli et al 1997). This investigation has evaluated a compound called poloxamer 407 (P-407) for its ability to function as a digestion inhibitor, specifically, as a lipase inhibitor, and effect weight loss in fat-fed mice.

P-407 is a triblock copolymer comprised of poly(oxyethylene) and poly(oxypropylene) units and functions as a nonionic surface-active-agent (surfactant). This agent has been shown to inhibit the biological activity of several lipases. In fact, to date, P-407 has been shown to inhibit the activity of hepatic lipase and lipoprotein lipase both in-vitro and in post-heparin plasma (Johnston & Palmer 1993; Wasan et al 2003), as well as endothelial lipase in-vitro (Johnston unpublished observations). Therefore, the purpose of this study was to determine whether P-407 would inhibit the activity of pancreatic lipase (PL) (triacylglycerol acylhydrolase; EC 3.1.1.3) and potentially function as an adjunct weight loss therapy similar to orlistat (tetrahydrolipstatin). If effective following oral administration to mice eating a high-fat diet, P-407 would prevent the hydrolysis of triglycerides to absorbable free fatty acids and monoglycerides, thereby mediating weight loss in these animals. Therefore, we first incubated pancreatic lipase with incrementally-increasing concentrations of P-407 in-vitro to determine the concentration at which 50% of the enzyme's activity was lost (IC50). Before the weight loss study, we evaluated whether inclusion of P-407 in the drinking water either induced diuresis or altered the volume of water ingested per day by changing the palatability of the water. An additional experiment was performed to quantify the amount of total lipids excreted into the faeces when mice were treated with P-407. Lastly, the effect of P-407 on weight loss in adult mice was assessed over a one-month period.

Materials and Methods

Materials

Porcine pancreatic lipase having an activity of 100 U mg^{-1} was obtained as a gift from Worthington Biochemical Corporation (Lakewood, NJ, USA). Olive oil, sodium chloride, sodium taurocholate, calcium chloride, sodium hydroxide, denatured ethanol, and petroleum ether were obtained from Sigma Chemical Co. (St Louis, MO, USA) and used as received. Male C57BL/6 mice (8–9-weeks old) were obtained from Charles River Laboratories (Wilmington, MA, USA). The high-fat mouse diet was obtained as a gift and consisted of the following ingredients: Purina mouse chow (5015) 750 g kg^{-1}, high-protein casein 75 g kg^{-1}, monohydrate dextrose 25 g kg^{-1}, sucrose 16.25 g kg^{-1}, dextrin 16.25 g kg^{-1}, cocoa butter 75 g kg^{-1}, cholesterol 12.5 g kg^{-1}, sodium cholate 5 g kg^{-1}, cellulose (fibre) 12.5 g kg^{-1}, mineral mix (AIN-76; 170915) 8.75 g kg^{-1}, vitamin mix (Teklad-40060) 2.5 g kg^{-1}, and choline chloride 1.25 g kg^{-1}. The percent of the diet by weight that was attributable to protein, carbohydrate, and fat was 19.7%, 45.7%, and 15.8%, respectively. The percent of kcal derived from the protein, carbohydrate, and fat in the diet was 19.4%, 45.3%, and 35.3%, respectively. The triglyceride-E test kit was obtained from Wako Chemicals, Inc. (Richmond, VA, USA).

Determination of pancreatic lipase activity in-vitro

The activity of PL was determined in the presence of incrementally-increasing concentrations of P-407 using the pH-stat method (Worthington 1988). The concentrations of P-407 evaluated were 2, 5, 10, 15, 20, 40, 80, and 160 μM. The assay for PL was a common titrimetric procedure utilized by Sigma Chemical Co. and published on their website. The principle of the assay is based on the hydrolysis of triglycerides to diglyceride and fatty acid in the presence of pancreatic lipase at 37 °C and a starting pH of 7.7. The release of fatty acids results in a reduction in the pH of the medium, the latter of which is maintained relatively constant by the addition of small volumes of sodium hydroxide throughout the timed assay. Olive oil served as the substrate in the assay.

The activity of PL was calculated for each P-407 concentration tested and the data expressed as the fraction of PL activity remaining vs the concentration of P-407 in the reaction medium. Each P-407 concentration was tested in triplicate for its ability to inhibit PL activity. The IC50 was estimated graphically from the line drawn through the experimental data points.

P-407 solution palatability and urine production

Before the one-month weight loss study, the palatability of the drinking water containing P-407 was assessed. This was necessary, because if P-407 imparted a pleasant taste to the drinking water, then mice might have ingested more water than normal. Increased water intake generally leads to a rather rapid initial weight loss, and therefore it could be erroneously concluded that any reduction in body weight observed was due to inhibition of PL by P-407 included in the drinking water. To test for this possibility, twelve male C57BL/6 mice aged between 55 and 63 days (average body weight 22.2 ± 0.2 g) were allowed to ingest either purified drinking water or water containing P-407. The twelve mice were each housed in a separate metabolism cage and were allowed to consume the high-fat chow and either purified water (controls; $n = 6$) or water containing 50 μM P-407 (treatment mice; $n = 6$) freely. This concentration of P-407 was selected based on the results of the in-vitro experiments which assessed the biological activity of PL as a function of P-407 concentration. Over the course of one week the volume of water ingested, the

amount of chow consumed, and the volume of urine produced per day were determined. Data from these measurements were then averaged for each group and compared for statistical significance using the Student's *t*-test.

The procedures for P-407 administration and blood collection were in accordance with the institution's guide for the care and use of laboratory animals, and the treatment protocol was approved by the Animal Care and Use Committee at the University of Missouri-Kansas City. All animals were treated in accordance with the NIH Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85-23, revised 1985).

One-month weight loss study

After a one-week wash-out period, the same 12 mice used in the study above were randomly divided into two groups. The average initial weight of the twelve mice was 23.1 ± 0.3 g. The control mice were allowed to consume the high-fat chow and purified water freely, whereas, the treatment mice were provided the same chow, but consumed water containing 50 μM P-407. The body weight of each mouse in both groups was determined daily over the course of one month. Data were then averaged for both groups of mice and presented as mean body weight vs time. The Student's *t*-test was used to determine whether mean values at each time point were significantly different between the control and P-407-treated mice.

Excretion of fat in faeces

The excretion of fat in the faeces of P-407-treated and control mice was determined using a gravimetric technique that measures total lipids (Wybenga & Inkpen 1974). Briefly, eight additional mice (average wt $= 23.0 \pm 0.2$ g) were randomly divided into two groups; P-407-treated and control. Both groups of four mice were provided the high-fat chow diet, which was freely available, beginning one day before the experiment. The control group received free access to water, while the P-407-treated mice were allowed to drink water containing P-407 at a concentration of 50 μM. For comparative purposes, a third group of mice was utilized in this experiment as well; namely, four mice that were allowed to consume standard, low-fat chow (fat content $\sim$4%) and water freely. Faeces were collected from all mice over the course of four days, and specimens from days 2 to 4 were pooled for determination of faecal total lipids.

Briefly, lipids were extracted from faeces by acidifying a known volume of a faecal emulsion, and then successively extracting the lipids with petroleum ether. The weight of the residue for each mouse in the P-407-treatment group which simultaneously consumed the high-fat chow was then averaged and compared with the corresponding mean values determined for mice contained in the high-fat chow and standard (low-fat) chow groups using a one-way analysis of variance. A Tukey's post hoc test was used to identify differences in the mean values if a significant ($P < 0.05$) *F* value was calculated from the analysis of variance. Mean values of faecal weight for the three treatment groups were also analysed for any significant differences using analysis of variance.

Plasma triacylglycerol concentrations after oral administration of a lipid emulsion

The concentration of plasma triacylglycerol following oral administration of a lipid emulsion was assessed in the presence and absence of P-407. Mice were fasted overnight and then administered, by oral gavage, either 0.5 mL of a lipid emulsion consisting of corn oil (3.0 mL), cholic acid (40 mg), and cholesterol oleate (1.0 g) plus physiological saline (3.0 mL) (n = 6) or the lipid emulsion (0.5 mL) plus P-407 (final concentration provided a dose of P-407 of 150 mg kg^{-1} of body weight) (n = 6). Using a heparinized capillary tube, blood samples (40 μL) were taken from the tail vein at 0.0, 0.5, 1.0, 1.5, 3.0, 4.0, and 5.0 h following administration of the lipid emulsion with or without P-407. The collected blood samples were centrifuged at 4 °C at 10 000 *g* for 10 min, the plasma harvested, and the plasma triacylglycerol concentration determined using the triglyceride test kit. Data were then averaged for both groups of mice and presented as the mean plasma triacylglycerol concentration vs time post-dosing. The Student's *t*-test was used to determine whether mean values at each time point were significantly different between the control and P-407-treated mice.

Results

In-vitro PL activity measurements

The results of these experiments are depicted in Figure 1. It can be noted that a typical sigmoidal log-concentration–response curve was observed, with approximately 90% PL activity abolished at a P-407 concentration of 28.4 μM. The IC50 was determined to be 15.9 μM.

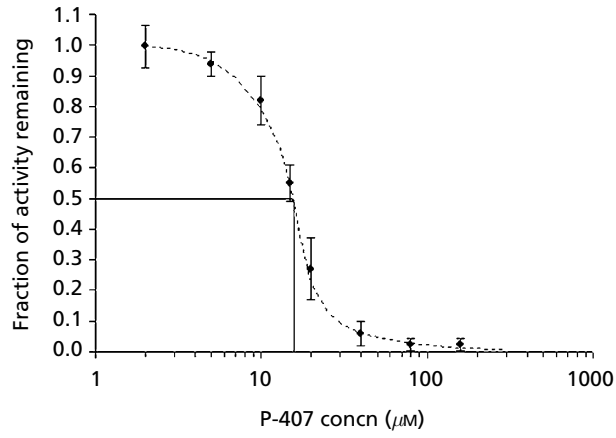

Figure 1 The effect of P-407 on the biological activity of pancreatic lipase in-vitro. The IC50 of 15.9 μM was estimated from the dashed line drawn through the data points. The dashed line does not represent a mathematical fit of the actual data points.

Effects of P-407 on intake and output

The data contained in Table 1 demonstrated that inclusion of P-407 50 μM in the drinking water had no effect on either the daily volume of water ingested, volume of urine produced, or the amount of chow consumed when the mean value of each parameter was compared with the corresponding mean value determined for controls.

P-407-mediated weight loss in mice

The average body weight vs time for each group of mice is depicted in Figure 2. It can be noted that beginning at day 10, the body weight of P-407-treated mice was significantly ($P < 0.05$) less than that observed for control mice. P-407-treated mice continued to demonstrate a reduction in overall body weight for the remainder of the study. By day 30, the average body weight of P-407-treated mice was 20.2 ± 1.1 g compared with 24.7 ± 0.9 g for controls. This represented an approximate 12% reduction in the initial body weight of P-407-treated mice over a 30-day period. Not unexpectedly, it can also be observed from Figure 2 that the control mice continued to gain weight throughout the 30-day study period; that is, increasing from an initial mean body weight of 23.1 ± 0.3 g to 24.7 ± 0.9 g by day 30 (an ~7.0% increase from initial body weight).

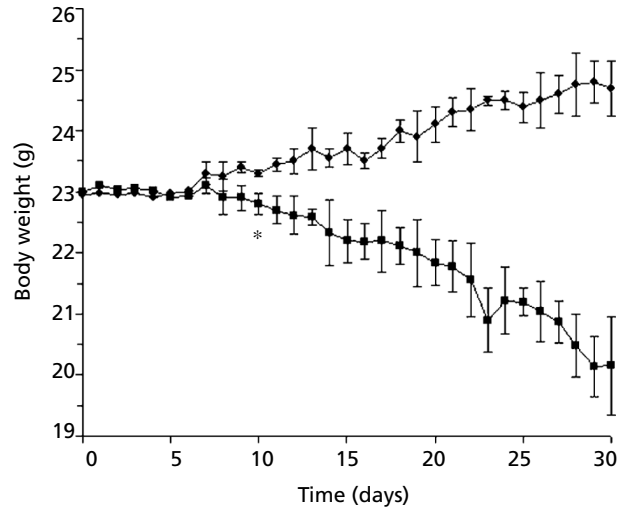

Figure 2 The effect of P-407 treatment on weight loss for mice consuming a moderately high-fat diet. ●, Control mice; ■, P-407-treated mice (150 mg kg^{-1}; based on an average of 5.6 mL water consumed per day and an average body weight of 23 g throughout the 30-day study). *$P < 0.05$, compared with control at day 10; all mean values of body weight for P-407-treated mice from day 10 onward were significantly ($P < 0.05$) less than the corresponding mean value for controls.

Faecal fat excretion

Table 2 lists the values of the average weight of faeces per day collected over days 2 to 4, as well as the weight of total lipids extracted from the faecal specimens, when mice were allowed to consume either a high-fat diet and purified water or a high-fat diet plus purified water containing P-407. As shown in Table 2, consumption of both the high-fat diet and the high-fat diet plus water containing P-407 resulted in a significant ($P < 0.05$) reduction in the average daily weight of the faeces when compared with mice eating a standard (low-fat) chow diet. However, the average daily weight of the faeces for mice ingesting the high-fat diet plus water containing P-407 was significantly ($P < 0.05$) greater than the mean value obtained for mice consuming the high-fat diet alone. The weight of total lipids in the faeces of mice which consumed the diet containing P-407 was significantly ($P < 0.05$) greater than the corresponding mean value for mice which consumed the high-fat diet only. Both groups of mice which consumed the high-fat diet, i.e. with or without P-407, had a significantly ($P < 0.05$) greater content of total lipids in the faeces than mice which ingested the standard, low-fat chow diet.

Table 1 The effect of P-407 on daily water ingested, urine produced, and chow consumed

Parameter	Purified water (controls)	Purified water + P-407 (50 μM)
Water ingested (mL)	5.72 ± 0.61 (6)[a]	5.56 ± 0.48 (6)
Urine produced (mL)	1.56 ± 0.09 (6)	1.60 ± 0.11 (6)
Chow consumed (g)	5.33 ± 0.52 (6)	5.43 ± 0.59 (6)

Values are mean ± s.d. [a]Number of animals.

Table 2 The effect of P-407 on the excretion of total lipids in faeces

Treatment	No. of mice	Faeces weight (g)	Total lipids in dry faeces (mg (g faeces)$^{-1}$)
Standard chow	4	1.3 ± 0.21	33 ± 1.9
High-fat chow	4	0.51 ± 0.042[a]	60 ± 3.8[a]
High-fat chow + P-407	4	0.63 ± 0.059[a,b]	87 ± 7.3[a,b]

[a]$P < 0.05$ compared with mice consuming a standard chow diet. [b]$P < 0.05$ compared with mice consuming the high-fat chow diet.

Plasma triacylglycerol concentrations after oral administration of a lipid emulsion

Figure 3 depicts the plasma triacylglycerol concentrations following oral administration of a lipid emulsion with or without P-407. While plasma concentrations of triacylglycerol for P-407-treated animals were, in general, lower than the corresponding plasma triacylglycerol concentrations for mice receiving the lipid emulsion only, statistically significant ($P < 0.05$) reductions were observed at

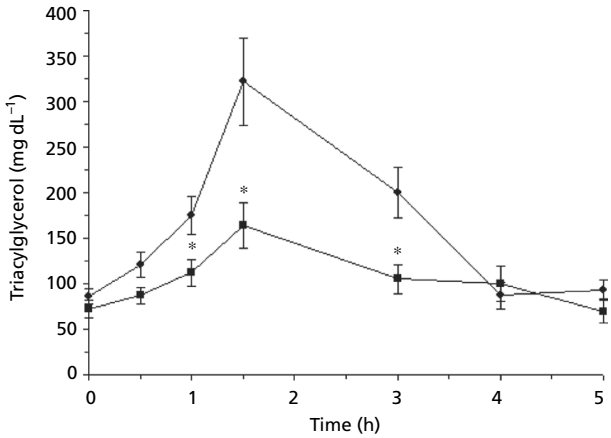

Figure 3 The effect of P-407 on the plasma concentration of triacylglycerol following oral administration of a lipid emulsion. ●, Control mice; ■, P-407-treated mice (150 mg kg^{-1}). *$P < 0.05$, significant decrease in the plasma triacylglycerol concentration relative to controls at the time points indicated.

only 1.0, 1.5, and 3.0 h post dosing. In addition, the plasma triacylglycerol concentration appeared to reach a maximum at approximately 1.5 h following administration of the lipid emulsions.

Discussion

Obesity is closely associated with life-style-related diseases such as hyperlipidaemia, hypertension, arteriosclerosis, and non-insulin-dependent diabetes mellitus and with increased risk of coronary heart disease. Thus, the present investigation was conducted to evaluate whether non-systemic administration of P-407 could aid in weight loss by inhibiting the activity of pancreatic lipase. Previously, P-407 had been shown to inhibit the biological activity of hepatic lipase and lipoprotein lipase both in-vitro and in post-heparin plasma (Johnston & Palmer 1993; Wasan et al 2003), as well as endothelial lipase in-vitro (Johnston unpublished observations), and so we hypothesized that P-407 may inhibit pancreatic lipase and induce weight loss similar to the marketed product called orlistat.

Orlistat is a covalent, active site-directed inhibitor of digestive lipases (Borgstrom 1988; Hadvary et al 1988) that reacts with the nucleophilic serine residue from the catalytic triad of pancreatic lipase (Hadvary et al 1991; Luthi-Peng et al 1992). By covalently blocking the lipase active site, orlistat inhibits the hydrolysis of dietary triglycerides and thus reduces the subsequent intestinal absorption of the lipolysis products monoglycerides and free fatty acids. Similar to the lipase-inhibiting effect of P-407, orlistat also inhibits the activity of lipoprotein lipase (Lookene et al 1994) and hormone-sensitive lipase (Smith et al 1996) in-vitro. The percentage of patients losing greater than 5% and greater than 10% of their body weight after one year of orlistat therapy (120 mg three times a day) was $44 \pm 8.4\%$ and $20 \pm 3.5\%$, respectively (Roche Technical Report 2003). Weight loss was typically observed within 14 days of initiation of orlistat therapy (Roche Technical Report 2003).

Our investigation demonstrated that P-407 was not as potent an inhibitor of pancreatic lipase activity as that reported for orlistat (Hogan et al 1987; Dollinger & Howell 1998; Roche Technical Report 2003). The IC50 of orlistat ranged from approximately 0.2 to 0.8 μM (Dollinger & Howell 1998), whereas the IC50 determined for P-407 in this study was 15.9 μM; a greater than 20-fold difference. Additionally, using mice which were orally administered a standard lipid emulsion containing orlistat at a dose of 45 mg kg^{-1}, Han et al (2005) demonstrated that plasma triacylglycerol concentrations were reduced to comparable levels seen in this study using P-407 at a dose of 150 mg kg^{-1}. Those same authors demonstrated that orlistat, at a dose of approximately 62 mg kg^{-1}/day, resulted in an 11-fold increase in the triacylglycerol excreted in the faeces compared with mice which consumed the high-fat diet without orlistat (Han et al 2005). While we neither measured faecal triacylglycerol content only, nor utilized orlistat in our studies, we did observe a statistically significant increase in the amount of total lipids excreted in the faeces of mice on a high-fat diet which were allowed free access to water containing P-407. Using a P-407 dose of 150 mg kg^{-1}/day, the amount of total lipids excreted in the faeces of mice fed the high-fat diet and which consumed water containing P-407 was approximately 45% greater than that observed for mice on the identical diet, but which ingested water without P-407. Therefore, P-407 was used at a dose that was 2.4-times greater than orlistat, but which resulted in only a 45% increase in faecal lipids, compared with the 11-fold increase in faecal triacylglycerol content observed by Han et al (2005) with orlistat (for reference purposes, triacylglycerol typically comprises ~10–15% of the total lipids in normal faeces (Wybenga & Inkpen 1974)).

The total lipids in the faeces of the mice used in this study could be compared with the total faecal lipids of man eating a non-high-fat diet. The average weight of faeces produced per day by man consuming a low-fat diet ranges from 69.8 to 128 g, with an average of 95 g (Sakata et al 2000). The total lipid content of faeces from normal individuals eating a standard (non-high-fat diet) ranges from 1 to 7 g/day (Wybenga & Inkpen 1974). Thus, total lipids normally comprise approximately 1.1%−7.4% of human faeces. In our study, the total lipid content of the faeces from mice eating a standard, low-fat chow diet was 3.3%. In contrast, the total lipid content of the faeces from mice eating the high-fat diet, while simultaneously consuming water without or with P-407, was 6.0% and 8.7%, respectively. Therefore, the figures for faecal fat (total lipids) excretion represented approximately 38% and 55% of the total fat content provided by ingestion of the high-fat chow (15.8% fat). For comparative purposes, another research group observed that mice fed a diet containing 21% w/w fat, 0.15% w/w cholesterol, and

orlistat (20 mg kg^{-1}/day) excreted 40% of the ingested fat in the faeces (Ueshima et al 2004). In other words, it required a dose of P-407 that was approximately 6-fold that of orlistat to excrete a comparable percent of ingested fat in the faeces (40% (Ueshima et al 2004) vs 55% (this study)).

Even more pertinent to the results of this study, Comai & Sullivan (1980) evaluated two other members of the same class of nonionic surface active agents as P-407 for their ability to inhibit PL and induce weight loss in rats. Those authors tested poloxamer 331 and poloxamer 188. The molecular weight of the hydrophobic poly(oxypropylene) core for poloxamers 188, 331, and 407 are 1750, 3250, and 4000, respectively, whereas the percent of the poly(oxyethylene) units in the molecule are 80%, 10%, and 70%, respectively. Thus, poloxamer 331 is a hydrophobic surface-active-agent, whereas both poloxamers 407 and 188 are more hydrophilic in nature. P-407 has 10% less poly(oxyethylene) units and has a greater percentage of poly(oxypropylene) units (as reflected by the larger molecular weight of the hydrophobe (polyoxypropylene)), and so P-407 is more hydrophobic than poloxamer 188. Comai & Sullivan (1980) demonstrated that the IC50 value of poloxamer 331 and 188 was 5.2 and 297 μM, respectively, compared with the value of 15.9 μM we determined for P-407. The lower value of the IC50 we obtained for P-407, compared with poloxamer 188, was probably due to the increased hydrophobic nature of P-407.

Comai & Sullivan (1980) administered both poloxamers to rats for 42 days at a dose of 420 or 1272 mg kg^{-1}/day (poloxamer 331) and 1320 mg kg^{-1}/day (poloxamer 188). They determined food consumed, body weight, fat content of the faeces, liver weight, carcass fat, and plasma cholesterol and triacylglycerol concentrations. With regard to body weight, those authors demonstrated that rats fed a diet containing poloxamer 331 at a dose of 420 or 1272 mg kg^{-1}/day and having a caloric density of 4.1 kcal g^{-1} resulted in a 62% and 90% reduction in weight gain, respectively, compared with controls, over the 42-day period. However, the reduction in weight gain for poloxamer 188 at a dose of 1320 mg kg^{-1}/day was only 14% and was not significantly different from controls. In this study, we determined that P-407 at a dose of 150 mg kg^{-1}/day (2.8- and 8.5-times less than the doses of poloxamer 331 tested) resulted in a 12% loss in initial body weight over 30 days. Interestingly, in this investigation and in the work reported by Comai & Sullivan (1980), administration of the poloxamer compounds did not alter the daily food consumption of the rodents.

Faecal fat elimination was also assessed by Comai & Sullivan (1980). Similar to this study, those authors used a gravimetric assay to measure faecal lipid content. Comai & Sullivan (1980) demonstrated that poloxamer 331 at a dose of 420 and 1272 mg kg^{-1}/day caused a significant increase of 50 mg/day (175% of control) and 125 mg/day (275% of control) in faecal fat excretion, respectively. In contrast, poloxamer 188 produced no significant increase in faecal fat elimination. Unfortunately, Comai & Sullivan (1980) did not report the weight of the faeces produced per day for the control and treated rats. In this study, the weight of the faeces produced per day was significantly less for mice consuming the high-fat diet, either with or without the inclusion of P-407, relative to controls. Additionally, mice consuming a high-fat diet and which consumed water containing P-407 had a significant increase in faecal lipids compared with both control mice and mice which consumed the high-fat chow and water without P-407. Similar to this study with P-407, Hogan et al (1987) and Ackroff & Sclafani (1996), using rats fed a high-fat diet containing orlistat (25 mg kg^{-1}/day), observed a reduction in body weight concomitantly with an increase in faecal fat excretion.

Conclusion

This study has demonstrated that P-407 was able to inhibit the activity of PL in-vitro and resulted in an approximate 12% loss in initial body weight over one month. Significant weight reduction in mice commenced 10 days after initiation of P-407 therapy, which was similar to the time period required for orlistat in man (approximately 14 days). Even though P-407 is more hydrophilic than poloxamer 331, its effectiveness, as assessed in this study, appeared to be comparable with poloxamer 331, especially in view of the fact that poloxamer 331 was utilized by Comai & Sullivan (1980) at doses that were 2.8- and 8.5-fold the dose of P-407 we employed. Although not as potent as orlistat, based on P-407's lack of toxicity, inability to be absorbed into the systemic circulation following oral administration, and rather low concentration (IC50 = 15.9 μM) required to inhibit the activity of PL, this agent may represent another potential digestion (lipase) inhibitor that could be used in conjunction with other weight-loss strategies, such as a proper diet and regular exercise, as well as with anti-obesity medications from other drug classes.

References

Ackroff, K., Sclafani, A. (1996) Effects of the lipase inhibitor orlistat on intake and preference for dietary fat in rats. *Am. J. Physiol.* **271**: R48–R54

Borgstrom, B. (1988) Mode of action of tetrahydrolipstatin: a derivative of the naturally occurring lipase inhibitor lipstatin. *Biochim. Biophys. Acta* **962**: 308–316

Bray, G. A. (1985) Obesity: definition, diagnosis, and disadvantages. *Med. J. Aust.* **142** (Suppl.): S2–S8

Bray, G. A. (1993) Use and abuse of appetite-suppressant drugs in the treatment of obesity. *Ann. Intern. Med.* **119**: 707–713

Cerulli, J., Lomaestro, B. M., Malone, M. (1997) Update on the pharmacotherapy of obesity. *Ann. Pharmacother.* **337**: 396–407

Comai, K., Sullivan, A. C. (1980) Antiobesity activity of pluronic L-101. *Int. J. Obes.* **4**: 33–42

Dollinger, L. M., Howell, A. R. (1998) A 2-methyleneoxetane analog of orlistat demonstrating inhibition of porcine pancreatic lipase. *Bioorg. Med. Chem. Lett.* **8**: 977–978

Garfinkel, L. (1985) Overweight and cancer. *Ann. Intern. Med.* **103**: 1034–1036

Hadvary, P., Lengsfeld, H., Wolfer, H. (1988) Inhibition of pancreatic lipase in vitro by the covalent inhibitor tetrahydrolipstatin. *Biochem. J.* **256**: 357–361

Hadvary, P., Sidler, W., Meister, W., Vetter, W., Wolfer, H. (1991) The lipase inhibitor tetrahydrolipstatin binds covalently to the putative active site serine of pancreatic lipase. *J. Biol. Chem.* **266**: 2021–2027

Han, L. K., Zheng, Y. N., Yoshikawa, M., Okuda, H., Kimura, Y. (2005) Anti-obesity effects of chikusetsusaponins isolated from Panax japonicus rhizomes. *BMC Compliment Alternative Med.* **5**: 1–10

Hogan, S., Fleury, A., Hadvary, P., Lengsfeld, H., Meier, M. K., Triscari, J., Sullivan, A. C. (1987) Studies on the anti-obesity activity of tetrahydrolipstatin, a potent and selective inhibitor of pancreatic lipase. *Int. J. Obes.* **11** (Suppl. 3): 35–42

Johnston, T. P., Palmer, W. K. (1993) Mechanism of poloxamer 407-induced hyper-triglyceridemia in the rat. *Biochem. Pharmacol.* **46**: 1037–1042

Lethbridge-Cejku, M., Schiller, J. S., Bernadel, L. (2004) Summary health statistics for U.S. adults: National Health Interview Survey, 2002. *Vital Health Stat.* **222**: 1–151

Lookene, A., Skottova, N., Olivecrona, G. (1994) Interactions of lipoprotein lipase with the active-site inhibitor tetrahydrolipstatin (Orlistat). *Eur. J. Biochem.* **222**: 395–403

Luthi-Peng, Q., Maerki, H. P., Hadvary, P. (1992) Identification of the active-site serine in human pancreatic lipase by chemical modification with tetrahydrolipstatin. *FEBS Lett.* **299**: 111–115

NIH Consensus Statement Covers Treatment of Obesity (1991) *Am. Fam. Phys.* **44**: 305–306

Pi-Sunyer, F. X. (1993) Medical hazards of obesity. *Ann. Intern. Med.* **119**: 655–660

Roche Pharmaceuticals, Inc. (2003) Complete product information for Xenical® (orlistat) capsules. Technical Report No. 27898691: 1–17

Sakata, Y., Ishigure, S., Shimbo, S. (2000) Weight of feces and its daily fluctuations in young women. Part I. A survey of the relation between fecal weight, dietary habits, and life-styles. *Nippon Koshu Eisei Zasshi* **47**: 385–393

Smith, G. M., Garton, A. J., Aitken, A., Yeaman, S. J. (1996) Evidence for a multi-domain structure for hormone-sensitive lipase. *FEBS Lett.* **396**: 90–94

Ueshima, K., Akihisa-Umeno, H., Nagayoshi, A., Takakura, S., Matsuo, M., Mutoh, S. (2004) A gastrointestinal lipase inhibitor reduces progression of atherosclerosis in mice fed a western-type diet. *Eur. J. Pharmacol.* **501**: 137–142

United States National Commission on Diabetes (1975) Report of the National Commission on Diabetes to the Congress of the United States. Bethesda (MD): U.S. Department of Health, Education, and Welfare. Publication No. 76-1021; Vol. 1

Van Itallie, T. B. (1985) Health implications of overweight and obesity in the United States. *Ann. Intern. Med.* **103**: 983–988

Wasan, K. M., Subramanian, R., Kwong, M., Goldberg, I. J., Wright, T., Johnston, T. P. (2003) Poloxamer 407-mediated alterations in the activities of enzymes regulating lipid metabolism in rats. *J. Pharm. Pharmaceut. Sci.* **6**: 108–116

Worthington, C. C. (1988) In: Worthington, C.C. (ed.) *Worthington enzyme manual.* Freehold: Worthington Biochemical Corporation, pp 212–214

Wybenga, D. R., Inkpen, J. A. (1974) Lipids. In: Henry, R. J., Cannon, D. C., Winkelman, J. W. (eds) *Clinical chemistry.* Harper & Row, New York, pp 1475–1482