Protocol: AAAR1622
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Title: Central Mechanisms and Predictors of Lorcaserin-Induced Weight Loss
Protocol Version #: 2
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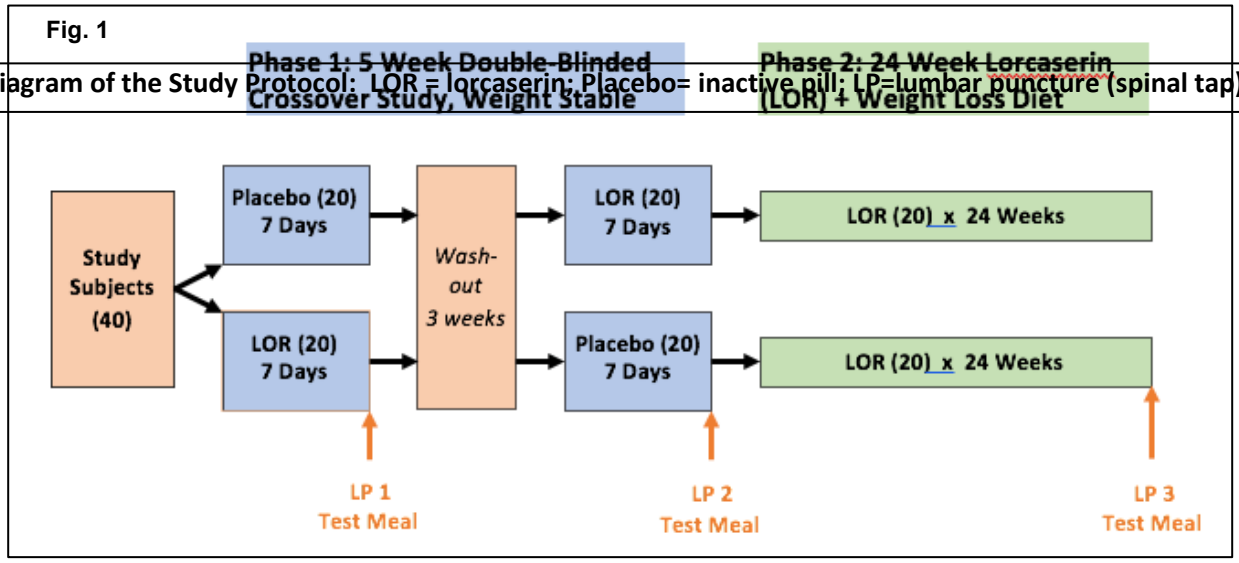
Study Design

40 healthy male and female subjects age 18-60 yr will be recruited for this study (BMI 28-40). Exclusion criteria include any clinically significant medical condition, including diabetes, uncontrolled hypertension, heart disease, bleeding disorder, kidney or liver dysfunction, neurologic disease, psychiatric or eating disorders; use of tobacco or opiates or history of alcohol or drug abuse; recent weight change ±5%; or medications that affect body weight. Subjects will have a physical examination and screening blood studies, including fasting glucose levels, comprehensive metabolic panel and CBC. An EKG will also be performed.

Fig. 1

Diagram of the Study Protocol: LOR = lorcaserin; Placebo= inactive pill; LP=lumbar puncture (spinal tap)



Subjects will be recruited for a 5-week crossover study with lorcaserin (LOR) and placebo followed by treatment with LOR or placebo for 24 weeks. **Phase 1:** Participants will be randomized to receive either LOR (10 mg bid) or placebo for 7 days followed by a 3 week washout period and will then be crossed over to receive 7 days of the other treatment (LOR or placebo). Thus each subject will be studied twice: once after receiving placebo and again after receiving lorcaserin. *Subjects will continue on a weight maintenance diet during this time to remain weight stable.* Salivary cortisol levels will be obtained at 0800h and at 2300h on days 2 and 6 of placebo and LOR. A 24h urine will be collected on day 6 of placebo and day 6 of LOR. On day 7 of both the placebo and LOR treatments, subjects will be studied in the clinical research center in the morning after an overnight fast. A lumbar puncture (spinal tap) will be performed and blood samples will be obtained. Subjects will then be fed a standardized breakfast (300 kcal). A laboratory test meal will be performed 4h later as described below. **Phase 2:** After completing the 7-day placebo and LOR studies, subjects will then be treated with LOR 10 mg bid (n= 40) for 24 weeks (Fig. 1). *Subjects will receive nutritional counseling to reduce their daily caloric intake to 600 kcal below their calculated caloric requirement and will be encourage to exercise moderately for 30 min daily.* Subjects will visit the clinic monthly during the 24 week study period for monitoring of vital signs and food counseling which will include review of food diaries. Salivary cortisol levels will be obtained the night before the monthly visits. Blood will be obtained at months 1, 2, 4 and 6 for safety monitoring and protein and hormone level analysis. At the end of the 24 week period subjects will be admitted to the GCRC for an LP followed by a fixed breakfast and a laboratory test meal 4h later. CSF will be tested for various proteins and hormones. Subjects will collect a 24h urine beginning the day before this final LP.

***Lumbar Puncture*:** LP will be performed after an overnight fast by Dr. Smiley, Professor of Anesthesia and Director of Obstetrical Anesthesia, using a 25G Whitacre needle. 12 ml of CSF (approximately 2 teaspoons) will be removed at each LP.

Laboratory Meal: Subjects will be studied in the Eating Behavior Lab under the supervision of Dr. Laurel Mayer. Subjects will be fed a standardized breakfast (300 kcal). A multiple-item test meal consisting of a fixed buffet will be performed 4h later. Subjects will be seated at a table in front of a food array and told to eat as much as they like over a 1h period. Hunger and satiety will be assessed using a visual analog scale. Food energy and macronutrient consumption will be calculated using the Nutrient Data Systems software program.

Study Purpose and Rationale

The prevalence of obesity and associated metabolic diseases continues to rise yet effective non-surgical long-term treatments remain elusive. The success of dieting can be enhanced by pharmacological treatment. However considerable individual variability is observed in response to pharmacotherapy and there is no way to predict who will respond well to a given medication. Peripheral metabolic signals, including leptin and insulin, communicate levels of energy stores to key brain regions and elicit a host of neuronal responses that maintain energy balance; such regulatory mechanisms make it difficult to maintain diet-induced weight loss. The overall research objective is to characterize these mechanisms and to determine how they are impacted by pharmacotherapy in order to predict who will or will not respond to a given weight loss medication. Studies will focus on the melanocortin neuronal system, consisting of the proopiomelanocortin (POMC)-derived MSH peptides, the MSH antagonist, agouti related protein (AgRP) and brain melanocortin receptors, that plays a critical role in responding to these peripheral signals and maintaining energy balance (1). This protocol will focus on the weight loss drug lorcaserin (LOR) a serotonin (5HT2c) receptor agonist, as it works primarily by activation of POMC neurons and brain melanocortin signaling. POMC neurons express 5-HT2cR and are activated by 5-HT agonists (2) In addition, the obesity phenotype of the global 5-HT2cR KO mouse can be normalized by selective 5-HT2cR expression only in POMC neurons (3). Furthermore the feeding effects of 5-HT agonists are abolished by genetic inactivation of either Pomc or the Mc4-r (4,5). Effects on glucose homeostasis also require activation of the Mc4-r (6). Thus a functional brain melanocortin circuit is required for 5-HT agonists to exert their effects on both energy balance and glucose metabolism. LOR decreases body weight in humans primarily by suppressing food intake without affecting energy expenditure (7-10). A pooled analysis of two randomized trials of 4008 and 3182 patients demonstrated that 47% of drug treated subjects achieved at least 5% wt loss (vs 23% for placebo) while 22% of subjects achieved at least 10% wt loss (vs 9% for placebo) (11). There were also differences in body fat, lipids and glycemic indicators. As with other drug therapies, there was a lot of individual variability in response to treatment such that 53% did not achieve clinically significant wt loss. There is evidence that greater than 5% wt loss at 12 wks can be used to predict the response to LOR at 1 yr (12). However given that there are now many choices for drug therapy it would be helpful to be able to predict long-term efficacy based on a biochemical profile or the acute feeding response to the drug.We propose to quantitate the extent to which POMC neurons are activated by LOR as a predictor of acute changes in feeding and long-term wt loss. The ability of acute changes in feeding behavior to predict long-term wt loss will also be studied. This approach has been used by our collaborator, Dr. Mayer, to demonstrate that the response to a laboratory meal after one wk treatment with phentermine was predictive of subsequent wt loss after 6 months of treatment (13). In addition to documenting the activation of hypothalamic POMC neurons it is also important to understand other neural or neuroendocrine consequences of drug treatment that would serve to attenuate the therapeutic effects of LOR. This protocol will focus on changes in the hypothalamic-pituitary-adrenal (HPA) axis and AgRP that could limit the effectiveness of LOR treatment. Serotonin agonists have well documented stimulatory effects on the HPA axis (14-17) but until recently the underlying mechanism has not been well

defined (18). The 5-HT2C R is highly expressed in CRH neurons in the hypothalamus and mediates the activation of these neurons by serotonin agonists with subsequent stimulation of pituitary ACTH and adrenal corticosterone release (18). The effects of LOR treatment on the HPA axis have not been reported. Given the potent stimulatory effects of glucocorticoids on food intake it is important to determine if treatment with LOR has acute and chronic effects on the HPA axis and if these effects impact the efficacy of drug treatment (19). We have previously found that measurements of intact POMC prohormone in cerebrospinal fluid (CSF) can serve as a marker of central POMC activity in humans; plasma AgRP has also emerged as a potential biomarker for brain AgRP. There are striking correlations of CSF POMC with BMI, adiposity, leptin and insulin; strong correlations of plasma AgRP with these parameters are also found. Furthermore, following short□term diet-induced weight loss, levels of CSF POMC and the POMC-derived peptide, ß-endorphin (ß-EP), decrease while CSF and plasma AgRP levels increase. We will therefore study the acute (1 wk) effects of LOR vs placebo on these biomarkers and on feeding behavior, in response to a laboratory test meal, as predictors of the longer-term (24-wk) response to LOR. Counterregulatory mechanisms that may limit the effectiveness of LOR treatment will be assessed by measurement of AgRP in CSF and plasma and of HPA activity. HPA activity will be characterized using plasma, salivary, urine and CSF measurements. The validation of biochemical markers (CSF and plasma neuropeptides) and behavioral markers (feeding response) that are predictive of drug efficacy would facilitate the choice of drug when initiating therapy. Furthermore understanding the counterregulatory responses that develop in response to weight loss and drug therapy could lead to interventions that improve drug efficacy.

Scientific Abstract

The overall objectives of this study are to validate biomarkers that can be used to predict responses to the weight loss medication, lorcaserin, and to characterize counterregulatory responses to drug therapy that may limit efficacy. Studies will focus on the hypothalamic melanocortin (MC) system, including the proopiomelanocortin (POMC)-derived MSH peptides, the MSH antagonist, agouti related protein (AgRP) and brain MC-Rs, which are key mediators of the central response to weight loss. Lorcaserin is a serotonin (5HT2cR) agonist that works primarily by activation of POMC neurons and melanocortin signaling. We have found that measurements of the POMC prohormone in cerebrospinal fluid (CSF) can serve as a marker of central POMC activity in humans; plasma AgRP measurements have also emerged as a potential biomarker of brain AgRP activity. The current protocol will examine the acute (1 wk) effects of lorcaserin vs placebo on these biomarkers and on feeding behavior, in response to a laboratory test meal, as predictors of the longer-term (24-wk) response to lorcaserin. Counterregulatory mechanisms that may limit the effectiveness of lorcaserin treatment will also be characterized with a focus on the hypothalamic□pituitary-adrenal (HPA) axis and changes in AgRP levels. 60 healthy male and female subjects age 18-60 yr (BMI 28-42) will be recruited for a 5-wk double-blind crossover study with lorcaserin and placebo followed by treatment with lorcaserin or placebo for 24 wks. Participants will be randomized to receive either lorcaserin or placebo for 7 days followed by a 3 wk washout period and will then be crossed over to receive 7 days of the other treatment. On day 7 of both the placebo and lorcaserin treatments, subjects will be studied in the morning after an overnight fast in the clinical research center. A lumbar puncture (LP) will be performed to obtain CSF and blood samples will be obtained. Subjects will then be fed a standardized breakfast (300 kcal). A laboratory test meal will be performed 4h later. After completing the 7 day placebo and lorcaserin studies, subjects will then be continued on lorcaserin (n= 40) for 24 wks. Subjects will receive nutritional counseling to reduce their daily caloric intake to 600 kcal below their calculated caloric requirement and will be encouraged to exercise moderately for 30 min daily. Subjects will visit the clinic monthly during the 24 wk study period for counseling and to review food diaries; vital signs and safely labs will be monitored. Blood will be obtained for AgRP and hormone levels. At the end of the 24 wk period subjects will be admitted to the clinical research center for an LP followed by a

fixed breakfast and a laboratory test meal 4h later. CSF and blood will be assayed for a melanocortin peptides and hormones. The validation of biochemical markers (CSF and plasma neuropeptides) and behavioral markers (feeding response) that are predictive of drug efficacy would facilitate the choice of drug when initiating therapy. Furthermore understanding the counterregulatory responses that develop in response to weight loss and drug therapy could lead to interventions that improve drug efficacy.

Statistical Procedures

40 subjects will be recruited to account for a predicted 20% dropout rate in order to retain 32 subjects. For phase 1, the primary outcome is difference in food intake after 7 days of drug compared to placebo. Based on known acute changes in food intake after LOR a sample size of 24 subjects would be required using paired analysis (10). This sample size is more than adequate if CSF POMC is used as the primary outcome variable using a SD of 59 fmol/m (for overweight and obese subjects) to detect a 20% change in CSF POMC as has been reported for hypothalamic POMC in animals after treatment with 5-HT2cR agonists (23). However in order to use the outcomes of phase 1 to predict phase 2 wt loss, 40 subjects will be recruited to retain 32 and to achieve a range of wt loss as predicted by clinical trials (9). Food intake, including food energy and macronutrient consumption will be correlated with baseline POMC and AgRP levels. Differences in food intake at the placebo and LOR test meals will be correlated with changes in melanocortin peptide levels as well as with wt loss after 24 wks. Multiple regression modeling will be used to predict phase 2 wt loss in the LOR-treated participants from the within-subject difference between LOR and placebo test meal results and CSF POMC measurements from Phase 1. Bivariate Pearson correlation coefficients will be determined and stepwise multiple regression analyses will be performed to detect associations between POMC and AgRP and changes in levels following LOR and placebo with relevant variables: wt change, food intake, adiposity, leptin, sOB-R, insulin, HOMA-IR, gut hormones. Phase 1 is a double-blind trial with lorcaserin and placebo. After 10 subjects completed phase 1 the investigators were unblinded to the treatment of those 10 subjects in order to perform an interim analysis on the neuropeptide changes. We did detect a significant change in CSF POMC levels in the first 10 subjects but changes were not significant for other POMC derived peptide values. We would now like to unblind the next 10 subjects who have completed phase 1 in order to perform an interim analysis on the first 20 subjects as this may help with determining if we need additional POMC-derived study markers and optimization of assay conditions. The double blind will then continue for the subsequent study subjects.

Data and Safety Monitoring

This study is considered to be of low risk to the participants but the risk is considered to be more than minimal risk as there is the potential risk as outlined above of post dural puncture headache. Subjects will be monitored for any adverse effect related to taking lorcaserin at all study visits over the 24 week period. CBC and metabolic panels will be obtained at monthly intervals for the first 2 month and then every 2 months for the remainder of the study. It is exceedingly unlikely that excess adverse events will occur and require stopping the study. However this will be carefully monitored by Drs. Wardlaw and Smiley and the Safety Ofcer, Dr. Freda. Each subject is evaluated for any adverse events. An adverse event is dened as both an expected side effect that is of a serious nature, or an unexpected side effect/event regardless of severity. All events will be graded as to their attribution (unrelated to protocol, or possibly, probably, or denitely related to protocol). Any event that is reported to either the principal investigator or her designated research associates by the subject or medical staff caring for the subject and which meets the criteria will be documented as such. All adverse events will be graded as mild, moderate, or severe. Any severe and/or unanticipated adverse event will be reported within 24h to the Safety Ofcer and IRB. All adverse events will be reported to the Safety Ofcer every 6 months. All adverse events will be summarized annually and submitted to the IRB.Unblinding ProcedureThe subject's safety takes priority

over any other considerations in determining if the treatment assignment should be unblinded. In the event of a medical emergency in which knowledge of the treatment is critical to a subject's management, the investigator may break the blind for the participant.