Impact of Timed Bromocriptine-QR Therapy upon Measures of Sympathetic Tone and Vascular Biology in Type 2 Diabetes Subjects

Statistical Analysis Plan
Date: Dec 14, 2018

Study Objectives:

Primary Objective:
To demonstrate the effects of dopaminergic activation with bromocriptine-QR on the autonomic nervous system in subjects with type 2 diabetes.

Secondary Objectives:
The Co-secondary objectives of the study were the following:

1) To assess the impact of bromocriptine-QR vs placebo on measures of insulin resistance and glycemic control
2) To demonstrate the effects of dopaminergic activation with bromocriptine-QR on the regulation of plasma neuroendocrine factors such as the hypothalamic-pituitary-axis (HPA) hormones, and on the plasma levels of markers of inflammation and oxidative/nitrosative stress in type 2 diabetes subjects.

Study Endpoints and Analysis Plan

Primary Endpoint

Changes in autonomic function measures from baseline to week 24 following treatment with study drug (bromocriptine-QR or placebo).

The effect of treatment with study drug for 24 weeks on the following three measures of autonomic function will be analyzed:

1) Heart Rate Variability
2) Resting Heart Rate
3) Sudorimetry

Between treatment group differences in the change from baseline to week 24 in the above autonomic function parameters will be analyzed. All analyses will be stratified by baseline resting heart rate (RHR) ≥70 or <70 beats per min (BPM) (see below). The rationale for such stratification is as follows. Elevated RHR is known to be a simple and useful clinical measure of elevated sympathetic nervous system activity as it reflects central sympathetic-parasympathetic activity balance[1,2] and has been shown to correlate with other measures of SNS activity such as muscle sympathetic nerve activity and serum noradrenalin levels and heart rate variability[3-6]. While clinically, RHR between 60-100 BPM is considered the "normal" range for RHR and RHR ≥100 is used as the criteria for defining tachycardia, a large body of evidence from epidemiological and clinical studies suggest that increasing RHR even within the "normal" range is associated with increased cardiometabolic risk, particularly above around 70, likely reflecting the deleterious effects of underlying elevated sympathetic tone leading to such elevated RHR. Such elevated RHR has been associated with insulin resistance[7,8], altered beta cell function[9], impaired glucose regulation and increased risk of developing type 2 diabetes mellitus[10-12] as well as increased cardiovascular risk[13-31] and mortality[13-23,26,27,29,31-40]. In addition to and consistent with the reported evidence supporting elevated RHR threshold of approximately 70 BPM as an indicator of elevated sympathetic tone, a previous study that evaluated the effect of bromocriptine-QR on RHR in subjects with type 2 diabetes demonstrated that bromocriptine-QR's influence to reduce RHR is only observable if the RHR is elevated to ≥ 70 BPM and the magnitude of this reduction was greater the more elevated the baseline RHR was above 70 BPM with greater reductions seen with baseline RHR ≥ 80[41,42]. Furthermore, it was observed that the degree of

bromocriptine-QR's impact to reduce elevated RHR was an independent predictor of its effect to reduce HbA1c among subjects with poor glycemic control (baseline HbA1c ≥ 7.5)[41]. The rationale for the categorical stratification of ≥70 or <70 BPM for the analysis of the data from this current study is therefore based on the above considerations. A subset analysis will also be conducted in subjects with baseline RHR ≥ 80 BPM. We hypothesize that the treatment effect on SNS will be expressed in the RHR ≥70 BPM group.

Secondary Endpoints:

1) Measures of insulin resistance and glycemic control

Given the mechanism of action of Cycloset to improve postprandial insulin sensitivity, the main endpoint that will be evaluated is the effect of study drug treatment on postprandial glycemic control based on the OGTT plasma glucose and insulin data obtained at baseline and week 24. Between treatment group differences in change from baseline to week 24 for the area under the curve as well as levels at different time points of OGTT plasma glucose and insulin levels will be analyzed. Matsuda index of insulin sensitivity calculated using the OGTT glucose and insulin levels and HOMA-IR calculated from fasting glucose and insulin levels will also be analyzed as measures of insulin sensitivity/resistance.

All subjects completing 24-weeks of study drug treatment with data for baseline and week 24 OGTT will be included in the analyses of glycemic endpoints. The analyses will be done in the entire cohort of subjects with and without stratification by baseline HbA1c level of <7.0 and ≥7.0 (i.e. suboptimal glycemic control).

Given that as a postprandial insulin sensitizer, the presence of adequate postprandial insulin levels is an important determinant of the postprandial glucose lowering efficacy, further subset analyses will be conducted to assess the above endpoints in subjects with OGTT insulin at T60 mins ≥50 μU/ml and also in the subset on an insulin secretory agent (+/- TZD). An analysis stratified by duration of diabetes (≤4 years or >4 years) may also be conducted depending on sample size.

2) Measures of inflammation, oxidative/nitrosative stress and plasma neuroendocrine factors

The following plasma factors will be evaluated: cortisol, aldosterone, norepinephrine, normetanephrine, adiponectin, leptin, IL6, TNF α, PAI1, SOD, TBARS, ADMA, nitrotyrosine and other appropriate factors of increased systemic inflammation and reactive oxygen species.

Between treatment group differences in the change from baseline to week 24 in the levels of the above factors will be analyzed in the overall cohort as well as with stratification by baseline RHR (</≥ 70 BPM) as described above for the analysis of autonomic function markers as well as stratification by baseline HbA1c (</≥ 7.0) as described above for the analysis of glycemic control parameters.

Statistical Methods:

Between treatment group (bromocriptine-QR vs. placebo) differences in change from baseline to week 24 and within group changes from baseline to week 24 of all primary and secondary endpoints will be analyzed using Independent Samples T-tests. If the data are not normally distributed in raw form or in some cases after simple log transformation, then appropriate non-parametric tests will be used instead. Within group analyses will be performed using paired samples T-test or repeated measures ANOVA as appropriate. The treatment effects on the primary and secondary outcomes will also be assessed after adjustment as needed for other potential factors that could influence the specific endpoint being analyzed such as duration of diabetes, baseline HbA1c, baseline RHR and/or change in RHR from baseline, concomitant medications and other baseline demographics as appropriate using categorical stratified analyses as discussed above and/or multivariable regression analyses methods.

References:

1. Cooley RL, Montano N, Cogliati C, van de Borne P, Richenbacher W, Oren R, Somers VK: Evidence for a central origin of the low-frequency oscillation in RR-interval variability. Circulation 1998;98:556-561
2. Monfredi O, Lyashkov AE, Johnsen AB, Inada S, Schneider H, Wang R, Nirmalan M, Wisloff U, Maltsev VA, Lakatta EG, Zhang H, Boyett MR: Biophysical characterization of the underappreciated and important relationship between heart rate variability and heart rate. Hypertension 2014;64:1334-1343.
3. Wulsin LR, Horn PS, Perry JL, Massaro JM, D'Agostino RB: Autonomic Imbalance as a Predictor of Metabolic Risks, Cardiovascular Disease, Diabetes, and Mortality. J Clin Endocrinol Metab 2015;100:2443-2448
4. Egan BM: Insulin resistance and the sympathetic nervous system. Curr Hypertens Rep 2003;5:247-254
5. Tentolouris N, Argyrakopoulou G, Katsilambros N: Perturbed autonomic nervous system function in metabolic syndrome. Neuromolecular Med 2008;10:169-178
6. Quarti Trevano F, Seravalle G, Macchiarulo M, Villa P, Valena C, Dell'Oro R, Mancia G, Grassi G: Reliability of heart rate as neuroadrenergic marker in the metabolic syndrome. J Hypertens 2017;35:1685-1690
7. Facchini FS, Stoohs RA, Reaven GM: Enhanced sympathetic nervous system activity. The linchpin between insulin resistance, hyperinsulinemia, and heart rate. Am J Hypertens 1996;9:1013-1017
8. Jiang X, Liu X, Wu S, Zhang GQ, Peng M, Wu Y, Zheng X, Ruan C, Zhang W: Metabolic syndrome is associated with and predicted by resting heart rate: a cross-sectional and longitudinal study. Heart 2015;101:44-49
9. Bonnet F, Empana JP, Natali A, Monti L, Golay A, Lalic K, Dekker J, Mari A, Balkau B, Group RS: Elevated heart rate predicts beta cell function in non-diabetic individuals: the RISC cohort. Eur J Endocrinol 2015;173:409-415
10. Shigetoh Y, Adachi H, Yamagishi S, Enomoto M, Fukami A, Otsuka M, Kumagae S, Furuki K, Nanjo Y, Imaizumi T: Higher heart rate may predispose to obesity and diabetes mellitus: 20-year prospective study in a general population. Am J Hypertens 2009;22:151-155
11. Bemelmans RH, Wassink AM, van der Graaf Y, Nathoe HM, Vernooij JW, Spiering W, Visseren FL, Group SS: Risk of elevated resting heart rate on the development of type 2 diabetes in patients with clinically manifest vascular diseases. Eur J Endocrinol 2012;166:717-725.
12. Aune D, B OH, Vatten LJ: Resting heart rate and the risk of type 2 diabetes: A systematic review and dose--response meta-analysis of cohort studies. Nutr Metab Cardiovasc Dis 2015;25:526-534
13. Bohm M, Reil JC, Deedwania P, Kim JB, Borer JS: Resting heart rate: risk indicator and emerging risk factor in cardiovascular disease. Am J Med 2015;128:219-228
14. Ho JE, Larson MG, Ghorbani A, Cheng S, Coglianese EE, Vasan RS, Wang TJ: Long-term cardiovascular risks associated with an elevated heart rate: the Framingham Heart Study. J Am Heart Assoc 2014;3:e000668
15. Fox K, Borer JS, Camm AJ, Danchin N, Ferrari R, Lopez Sendon JL, Steg PG, Tardif JC, Tavazzi L, Tendera M, Heart Rate Working G: Resting heart rate in cardiovascular disease. J Am Coll Cardiol 2007;50:823-830

16. Tadic M, Cuspidi C, Grassi G: Heart rate as a predictor of cardiovascular risk. Eur J Clin Invest 2018;48
17. Caetano J, Delgado Alves J: Heart rate and cardiovascular protection. Eur J Intern Med 2015;26:217-222
18. Cooney MT, Vartiainen E, Laatikainen T, Juolevi A, Dudina A, Graham IM: Elevated resting heart rate is an independent risk factor for cardiovascular disease in healthy men and women. Am Heart J 2010;159:612-619 e613
19. Arnold JM, Fitchett DH, Howlett JG, Lonn EM, Tardif JC: Resting heart rate: a modifiable prognostic indicator of cardiovascular risk and outcomes? Can J Cardiol 2008;24 Suppl A:3A-8A
20. Bohm M, Swedberg K, Komajda M, Borer JS, Ford I, Dubost-Brama A, Lerebours G, Tavazzi L, Investigators S: Heart rate as a risk factor in chronic heart failure (SHIFT): the association between heart rate and outcomes in a randomised placebo-controlled trial. Lancet 2010;376:886-894
21. Fox K, Bousser MG, Amarenco P, Chamorro A, Fisher M, Ford I, Hennerici MG, Mattle HP, Rothwell PM, Investigators PS: Heart rate is a prognostic risk factor for myocardial infarction: a post hoc analysis in the PERFORM (Prevention of cerebrovascular and cardiovascular Events of ischemic origin with teRutroban in patients with a history oF ischemic strOke or tRansient ischeMic attack) study population. Int J Cardiol 2013;168:3500-3505
22. Fox K, Ford I, Steg PG, Tendera M, Robertson M, Ferrari R, investigators B: Heart rate as a prognostic risk factor in patients with coronary artery disease and left-ventricular systolic dysfunction (BEAUTIFUL): a subgroup analysis of a randomised controlled trial. Lancet 2008;372:817-821
23. Tverdal A, Hjellvik V, Selmer R: Heart rate and mortality from cardiovascular causes: a 12 year follow-up study of 379,843 men and women aged 40-45 years. Eur Heart J 2008;29:2772-2781
24. Nikolovska Vukadinovic A, Vukadinovic D, Borer J, Cowie M, Komajda M, Lainscak M, Swedberg K, Bohm M: Heart rate and its reduction in chronic heart failure and beyond. Eur J Heart Fail 2017;19:1230-1241
25. Swedberg K, Komajda M, Bohm M, Borer JS, Ford I, Dubost-Brama A, Lerebours G, Tavazzi L, Investigators S: Ivabradine and outcomes in chronic heart failure (SHIFT): a randomised placebo-controlled study. Lancet 2010;376:875-885
26. Woodward M, Webster R, Murakami Y, Barzi F, Lam TH, Fang X, Suh I, Batty GD, Huxley R, Rodgers A, from the Asia Pacific Cohort Studies C: The association between resting heart rate, cardiovascular disease and mortality: evidence from 112,680 men and women in 12 cohorts. Eur J Prev Cardiol 2014;21:719-726
27. Diaz A, Bourassa MG, Guertin MC, Tardif JC: Long-term prognostic value of resting heart rate in patients with suspected or proven coronary artery disease. Eur Heart J 2005;26:967-974
28. Khan H, Kunutsor S, Kalogeropoulos AP, Georgiopoulou VV, Newman AB, Harris TB, Bibbins-Domingo K, Kauhanen J, Gheorghiade M, Fonarow GC, Kritchevsky SB, Laukkanen JA, Butler J: Resting heart rate and risk of incident heart failure: three prospective cohort studies and a systematic meta-analysis. J Am Heart Assoc 2015;4:e001364
29. Wang SL, Wang CL, Wang PL, Xu H, Du JP, Zhang DW, Gao ZY, Zhang L, Fu CG, Chen KJ, Shi DZ: Resting heart rate associates with one-year risk of major adverse cardiovascular events in patients with acute coronary syndrome after percutaneous coronary intervention. Exp Biol Med (Maywood) 2016;241:478-484

30. Castagno D, Skali H, Takeuchi M, Swedberg K, Yusuf S, Granger CB, Michelson EL, Pfeffer MA, McMurray JJ, Solomon SD, Investigators C: Association of heart rate and outcomes in a broad spectrum of patients with chronic heart failure: results from the CHARM (Candesartan in Heart Failure: Assessment of Reduction in Mortality and morbidity) program. J Am Coll Cardiol 2012;59:1785-1795
31. Vazir A, Claggett B, Cheng S, Skali H, Shah A, Agulair D, Ballantyne CM, Vardeny O, Solomon SD: Association of Resting Heart Rate and Temporal Changes in Heart Rate With Outcomes in Participants of the Atherosclerosis Risk in Communities Study. JAMA Cardiol 2018;3:200-206
32. Bemelmans RH, van der Graaf Y, Nathoe HM, Wassink AM, Vernooij JW, Spiering W, Visseren FL, Group SM: The risk of resting heart rate on vascular events and mortality in vascular patients. Int J Cardiol 2013;168:1410-1415
33. Custodis F, Roggenbuck U, Lehmann N, Moebus S, Laufs U, Mahabadi AA, Heusch G, Mann K, Jockel KH, Erbel R, Bohm M, Mohlenkamp S: Resting heart rate is an independent predictor of all-cause mortality in the middle aged general population. Clin Res Cardiol 2016;105:601-612
34. Greenland P, Daviglus ML, Dyer AR, Liu K, Huang CF, Goldberger JJ, Stamler J: Resting heart rate is a risk factor for cardiovascular and noncardiovascular mortality: the Chicago Heart Association Detection Project in Industry. Am J Epidemiol 1999;149:853-862
35. Alhalabi L, Singleton MJ, Oseni AO, Shah AJ, Zhang ZM, Soliman EZ: Relation of Higher Resting Heart Rate to Risk of Cardiovascular Versus Noncardiovascular Death. Am J Cardiol 2017;119:1003-1007
36. Disegni E, Goldbourt U, Reicher-Reiss H, Kaplinsky E, Zion M, Boyko V, Behar S: The predictive value of admission heart rate on mortality in patients with acute myocardial infarction. SPRINT Study Group. Secondary Prevention Reinfarction Israeli Nifedipine Trial. J Clin Epidemiol 1995;48:1197-1205
37. Nauman J, Janszky I, Vatten LJ, Wisloff U: Temporal changes in resting heart rate and deaths from ischemic heart disease. JAMA 2011;306:2579-2587
38. Kristal-Boneh E, Silber H, Harari G, Froom P: The association of resting heart rate with cardiovascular, cancer and all-cause mortality. Eight year follow-up of 3527 male Israeli employees (the CORDIS Study). Eur Heart J 2000;21:116-124
39. Gillman MW, Kannel WB, Belanger A, D'Agostino RB: Influence of heart rate on mortality among persons with hypertension: the Framingham Study. Am Heart J 1993;125:1148-1154
40. Pittaras AM, Faselis C, Doumas M, Myers J, Kheirbek R, Kokkinos JP, Tsimploulis A, Aiken M, Kokkinos P: Heart rate at rest, exercise capacity, and mortality risk in veterans. Am J Cardiol 2013;112:1605-1609
41. Chamarthi B, Vinik AI, Ezrokhi M, Cincotta AH. Dopamine agonist therapy reduces elevated heart rate and dysglycemia in Type 2 diabetes subjects. Diabetes. 2016;65(Suppl1):A317
42. Chamarthi B, Ezrokhi M, Cincotta AH. Bromocriptine-Quick Release (BQR) Reduces Elevated Sympathetic Tone and Hyperglycemia in Type 2 Diabetes (T2D). Diabetes. 2017;66(Suppl1):A348