Exercise hemodynamic and neurohormone responses as sensitive biomarkers for diltiazem in rats

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ABSTRACT-Purpose. To investigate the potential of exercise hemodyanamic and neurohormone variables as sensitive biomarkers for pre-clinical evaluation of diltiazem (DTZ). **Methods.** Sprague Dawley (SD) rats were randomly divided into 3 groups (n = 6 - 8 each), and each group received DTZ 10 mg/kg twice daily for 5 doses or saline followed by a treadmill exercise protocol for 7 min with speed set at 7 m/min at 3 % grade. The 3rd group received saline but no exercise. **Results.** Exercise increased SBP from 108 ± 2 to 131 ± 3 mmHg, and HR from 437 ± 6 to 503 ± 6 bpm, and plasma epinephrine concentrations from 2.0 ± 0.6 to 5.8 ± 1.7 ng/mL in control rats ($p < 0.05$ for all variables), but had no significant effect on DBP (81 ± 5 vs 87 ± 6 mmHg) and plasma norepinephrine concentrations (1.5 ± 0.2 vs 3.9 ± 0.4 ng/mL). The hemodynamic responses to exercise were significantly attenuated by DTZ ($p < 0.05$), but the effect on neurohormone response was minimal ($p > 0.05$). **Conclusion.** Exercise hemodynamic and neurohormone responses are sensitive biomarkers which could be used for safety and efficacy evaluation of DTZ and perhaps also other calcium antagonists in pre-clinical animal models.

INTRODUCTION

Biomarkers are increasingly used in drug discovery and development, and use of biomarkers are widely considered as the scientific basis for targeted therapy and personalized medicine (1-5).

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Although diltiazem (DTZ) which is a calcium antagonist widely used in the treatment of angina and hypertension (6, 7), it also has anti-platelet properties (8) and considerable potential for prevention of atherosclerosis and stroke (9). It is extensively metabolised yielding a host of metabolites some of which have potent pharmacological activities (10, 11). In addition, many calcium antagonists have been shown to inhibit the uptake of adenosine by erythrocytes (RBC) *in vitro*, and that some of the metabolites are considerably more potent than the parent DTZ (11 , 12).

For many years the safety of calcium antagonists particularly for the short acting dihydropyridines have been a cause for concern in post MI patients and those with congestive heart failure (13). It is believed that sympathetic activation is an important pathologic mechanism for many cardiovascular diseases (14, 15), and a probable cause of serious adverse cardiac events associated with some of the short-acting calcium antagonists (16). Most calcium antagonists increase plasma concentrations of norepinephrine after chronic use (16), although clinical significance of these observations has not been clearly demonstrated (14). We have shown that exercise hemodynamic variables are better predictors of pharmacodynamic response to DTZ than ambulatory variables (17), but such pharmacokinetics/hemodynamic relationships in exercise are often difficult to be demonstrated in patients because of factors related to heterogeneity of cardiovascular diseases and poly-pharmacy issues in the patient population. A working pre-clinical exercise animal model simulating neurohormone activation would obviate many of these difficulties and would be useful for proof of concept studies for safety and efficacy evaluation. This paper evaluates the potential of exercise hemodynamic and neurohormone responses as more sensitive pre-clinical biomarkers for cardiovascular effects of diltiazem using an exercise rat model which have not been reported.

METHODS

Chemicals

DTZ was kindly received as gift from Biovail Corp (Mississauga, Ont., Canada). Catecholamines were

purchased from Sigma Chem. Co. (St. Louis, MO, U.S.A.). Solvents were HPLC grade (BDH Chem., Halifax, NS, Canada), and all other chemicals were reagent grade (Fisher Scientific, Ont., Canada).

Study protocol

The study protocol was approved by the Dalhousie University Committee on Laboratory Animals (UCLA), and following the guidelines established by the Canadian Council for Animal Care. Male Sprague-Dawley (SD) rats, weighing between 440 - 530 g were purchased from Charles River Laboratories (Montreal, QC, Canada). They were randomly divided into 3 groups (n = 6 - 8 each). An indwelling silastic catheter (0.020" ID x 0.037" OD, Dow Corning Corp., MI, USA) was implanted into the right carotid artery of each animal under general anaesthesia as described previously (18). After recovery from the surgery (24 h), each animal received either normal saline (Control A) or 10 mg/kg of DTZ given by subcutaneous injection twice daily for 5 doses, prior to the exercise experiment. The 3rd group received normal saline by the same administration route but no exercise (Control B).

The exercise test was performed on a research Exercise Treadmill (Model Exer-4, Columbus Instruments International Corporation, Columbus, Ohio, U.S.A.), with speed set at 7 m/min at 3 % grade. Each rat was exercised under this condition for 7 minutes. Hemodynamic variables were recorded by a Tektronix 400 monitor with chart speed set at 25 mm/sec and range of the pressure transducer at 150 mmHg (Tektronix 414 Portable Patient Monitor & 400 Medical Recorder, Tektronix, Inc., Beaverton, Oregon, USA).

Blood samples (0.5 mL) were obtained from the carotid artery before DTZ, at 0 (before exercise), 5 and 7 minutes after onset of exercise, and 5, 30 and 60 minutes after completion of exercise for measurement of catecholamines. In addition, hemodynamic recordings (HR, SBP, DBP) were obtained from the right carotid artery throughout the exercise experiment. Plasma concentrations of catecholamines were determined by a previously reported HPLC (19 , 20).

Data analysis

Hemodynamic and neurohormone data between groups were analyzed by ANOVA and difference considered significance when $p < 0.05$. Differences within the same animal before and after exercise, or before and after DTZ were evaluated by ANOVA followed by the Dunnett test and considered significant at $p < 0.05$ (Minitab®, Minitab Inc., Release 14, State College, PA, USA). Data are presented as mean ± SEM.

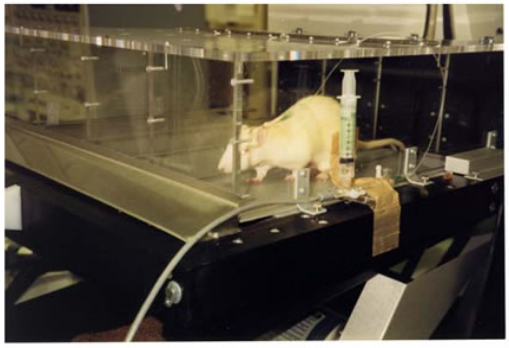
RESULTS

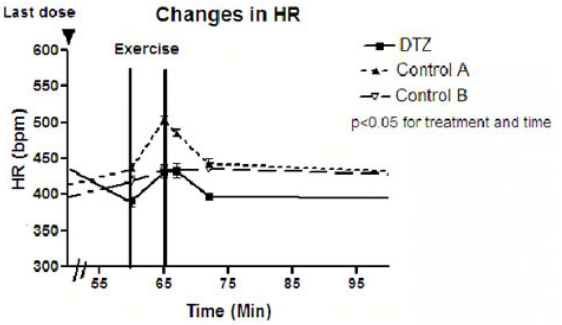
DTZ given at 10 mg/kg s.c. twice daily for 5 doses was well tolerated by the animals which were able to complete fully the described exercise test. At 1 hour after the last dose, it decreased SBP before exercise from 115 ± 1 to 96 ± 2 mmHg (-17%), DBP from 97 ± 2 to 70 ± 2 mmHg (-28%), and HR from 439 ± 5 to 391 ± 9 bpm (11%) ($p < 0.05$ for all variables), but had minimal effect on plasma concentrations of norepinephrine (1.4 ± 0.3 vs 1.9 ± 0.9 ng/mL), or epinephrine (0.76 ± 0.11 vs 0.84 ± 0.10 ng/mL) ($p > 0.05$). Exercise increased SBP from 108 ± 2 to 131 ± 3 mmHg (21%), and HR from 437 ± 6 to 503 ± 6 bpm (15%), and plasma epinephrine concentrations from 0.94 ± 0.13 to 5.8 ± 1.7 ng/mL in control rats ($p < 0.05$ for all variables), but had less effect on DBP (81 ± 5 vs 87 ±6mmHg) & plasma norepinephrine concentrations (1.5 ± 0.2 vs 3.9 ± 0.4 ng/mL). In DTZ treated rats, SBP was increased from 96 ± 2 to 103 ± 2 mmHg (7%), HR from 391 ± 9 to 433 ± 9 (11%) ($p < 0.05$), and epinephrine and norepinephrine from 0.84 ± 0.1 to 4.7 ± 1.9 ng/mL, and 1.9 ± 0.3 to 5.1 ± 1.9 ng/mL, respectively.The increases in catecholamine concentrations attributed to DTZ were not significantly different from the increase due to exercise. In contrast to the slight increase in the control rats, DBP was decreased from 70 ± 2 to 64 ± 4mmHg(-10%) during exercise in the DTZ treated rats, and continue to decrease immediately post exercise(Fig.1).The neurohormone & hemodynamic variables gradually returned to pre-exercise level shortly after exercise (Figures 1 and 2).

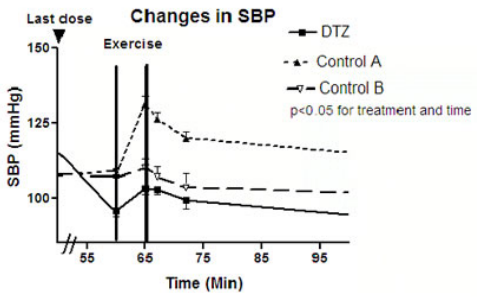
Table 1. Hemodynamic and neurohormone effects of diltiazem in rats during exercise after 10 mg/kg sc bid for 5 doses[a].

Neurohormones	Treatment/Control[b]	Pre-Exercise	Maximum Exercise	Post-Exercise	Treatment and exercise effect by ANOVA
Norepinephrine (ng/mL)	Control A	1.5 ± 0.2	3.9 ± 1.4	1.0 ± 0.2	P > 0.05
	Control B	1.1 ± 0.2	1.1 ± 0.2	1.9 ± 0.4	
	DTZ	1.9 ± 0.3	5.1 ± 1.9	1.8 ± 0.3	
Epinephrine (ng/mL)	Control A	0.94 ± 0.13	5.8 ± 1.7	1.8 ± 0.6	P < 0.05 for treatment
	Control B	0.86 ± 0.19	1.4 ± 0.3	1.0 ± 0.3	
	DTZ	0.84 ± 0.10	4.7 ± 1.9	2.3 ± 0.3	
SBP (mmHg)	Control A	108 ± 2	131 ± 3	115 ± 2	P < 0.05
	Control B	107 ± 3	107 ± 3	102 ± 3	
	DTZ	96 ± 2	103 ± 2	94 ± 3	
DBP (mmHg)	Control A	81 ± 5	87 ± 6	76 ± 7	P< 0.05 for treatment
	Control B	90 ± 1	88 ± 2	85 ± 3	
	DTZ	70 ± 2	64 ± 4	69 ± 3	
HR (bpm)	Control A	437 ± 6	503 ± 6	431 ± 11	P < 0.05
	Control B	419 ± 8	434 ± 9	428 ± 10	
	DTZ	391 ± 9	433 ± 9	394 ± 10	

[a] Measurements were obtained within 0.5 hr before and post exercise . Each value represents mean ± SEM of 5 – 6 animals. [b] Control A – rats received saline and exercise; Control B – rats received saline but no exercise.







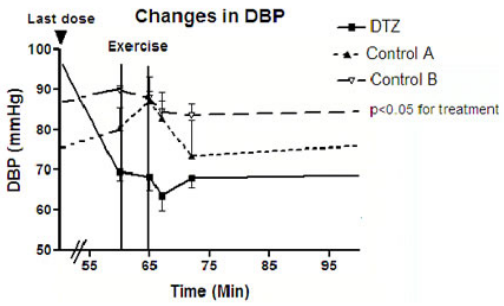


Figure 1. Hemodynamic responses to exercise in control and DTZ treated rats (Values are mean ± SEM). The vertical boundary represents the exercise period (7 min).

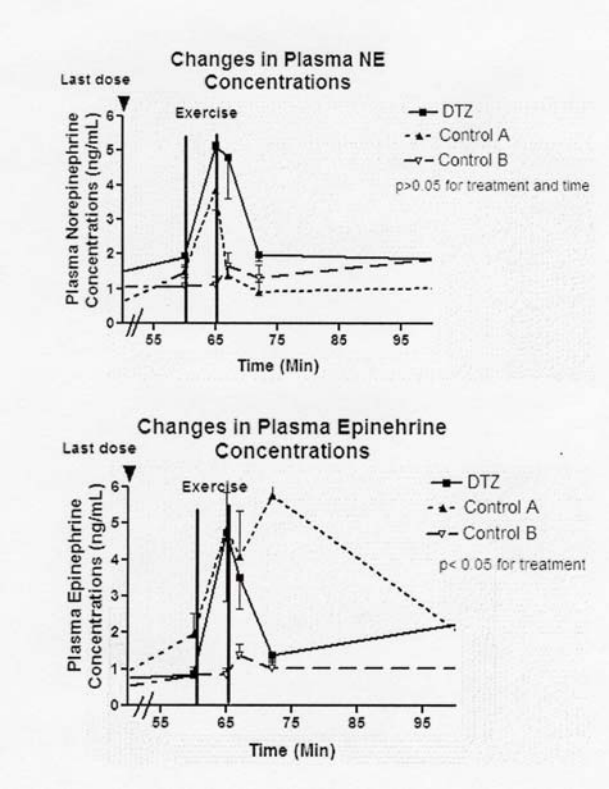


Figure 2. Neurohormone responses to exercise in control and DTZ treated rats (Values are mean ± SEM). The vertical boundary represents the exercise period (7 min).

Changes were minimal in the control rats without exercise (Control B), and the results are summarised in Table 1. Plasma concentrations of other catecholamines (e.g. dopamine and DOPAC) were higher in the DTZ treated rats, but not affected by exercise (unpublished).

DISCUSSION

Previous studies in rats have shown that hemodynamic variables changed during the course of an experiments whether the animals were kept in a restrainer or allowed to move freely (21). Thus it was necessary to employ two control groups: one group received saline and exercise (Control A) and the other received saline but no exercise (Control B). Using this experimental design, effects attributed to DTZ and exercise were statistically significant between the three treatment groups for the hemodynamic variables studied and plasma concentrations of epinephrine. However, due to the small number of animal used (6 per each group) and the large inter-individual differences observed particularly for the neurohormone variables (CV > 50%), the effects observed for the changes in norepinephrine concentrations did not reach statistically significant level.

Previous studies have shown that a single 20 mg/kg dose of DTZ administered systemically decreased blood pressure (SBP and DBP) and heart rate (HR) in normotensive rats, and that the effects were greater on the DBP than the SBP (-50% vs –20%) (21 , 22). A similar hemodynamic effect of DTZ was also evident in the current study when it was administered subcutaneously at 10 mg/kg twice daily for 5 doses although the effects were significantly smaller (-28% vs -17%). These differences could be attributed to the different plasma concentrations of DTZ and its active metabolites attained after the two different dosages and mode of administration (21). The current study has also demonstrated that exercise increased BP, HR, and plasma concentrations of epinephrine in rats (Table 1, Figures 1 and 2) which are consistent with the previous results reported by other workers (23). The fact that DTZ lowered BP and HR, but had no effect on the increase of plasma concentrations of norepinephrine and epinephrine, prior to and in response to exercise, would suggest that it has minimal effect on neurohormone or sympathetic activation, and that there is no synergistic effect between exercise and DTZ as was demonstrated for cocaine in the study by these workers (23). An earlier study of DTZ using a congestive heart failure dog model by right ventricular pacing has shown that a 0.8 mg/kg dose of DTZ increased plasma concentrations of norepinephrine induced by pacing without affecting the HR response (24). The reasons for the discrepancy is not clear although it may be related to the different animal models employed and that sympathetic activation was induced by a different mechanism (exercise vs ventricular pacing). Clinically, it has been documented that chronic use of short acting dihydropyridine calcium antagonists (e.g. nifedipine) increases ambulatory plasma norepinephrine concentrations which correlate with changes in hemodynamic variables. On the other hand, long acting calcium antagonists such as

amlodipine have minor effects or actually decrease plasma norepinephrine concentrations, although there were considerable discrepancies among these clinical studies. The effect of the calcium antagonists with negative chronotropic effects such as diltiazem and verapamil is not clear (16 , 25). Thus there are many factors attributing to the hemodynamic and neurohormone effects of calcium antagonists, and it is not clear if ambulatory plasma norepinephrine concentrations are useful biomarkers for therapeutic monitoring (14). We have shown that DTZ attenuated the hemodynamic responses to exercise in healthy volunteers and in patients with effort angina (26, 27), and more recently that exercise variables are better predictors of the hemodynamic effects than resting variables (17). Using the described exercise animal model, the current study has demonstrated that while DTZ attenuated the ambulatory hemodynamic response from 10 % for the HR to 28% for DBP, the effects on the exercise responses were so much greater such that a reduction of an increase of SBP from 23 to 7 mmHg (-70%), DBP from +6 to –6 mmHg (-200%), and HR from 66 to 42 bpm (-36%) were observed. It is clear from this animal experiment and from previous clinical studies (26 , 28) that exercise increased SBP more than DBP (21 vs 7%) and that diltiazem has greater effect on lowering DBP than SBP (-28 vs -17%). It is known that clinically blood pressure tends to be lower following exercise than before exercise (29 , 30), which may explain the beneficial effect of exercise for hypertension (31). A similar post-exercise hypotension was also observed in the current exercise rat model (Table 1 and Figure 1). As a result of these and perhaps other factors as well, diltiazem completely suppressed the increase of DBP during exercise, and exacerbating the post-exercise hypotension in the drug treated rats.

Although the mechanism of post-exercise hypotension is not clear, it could be related to an hormonal interactions involving adenosine and neurohormones. Adenosine is an endogenous vasodilator and a key mediator for ischemia preconditioning and cardiac protection (32 , 33). It has been shown previously that many calcium antagonists inhibit the uptake of adenosine by erythrocytes and other cell types (12 , 34). More recently, we have shown that DTZ inhibits the oxidative metabolism of adenosine in healthy volunteers and patients with effort angina (35), and potentiates the hemodynamic effects of exogenous adenosine in rabbits (36). These evidences indicate that DTZ may potentiate the cardiovascular effects of adenosine during exercise which is known to have an inhibitory effect on the sympathetic control of the hemodynamic variables in the body (37). Further studies are needed to investigate the mechanisms involved in these neurohormonal interactions. In conclusion, we have demonstrated that exercise hemodynamic variables and neurhormone concentrations determined from the described pre-clinical model are relevant mechanistic biomarkers which are more sensitive than ambulatory measurement. These biomarkers could be used to probe the inherent safety and efficacy of calcium antagonists and perhaps also for other classes of cardiovascular agents.

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