THE EFFECT OF CAPTOPRIL ON BLOOD GLUCOSE, PLASMA INSULIN AND BLOOD PRESSURE VIA A NITRIC OXIDE-INDEPENDENT MECHANISM IN AN ANIMAL MODEL

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INTRODUCTION

Hypertension and diabetes mellitus coexist more frequently than it would be estimated from their relative prevalence in the general population (1). Hypertension in diabetic patients is usually essential (2) and mild to moderate but rarely malignant (3), occurring twice as often in diabetic as in nondiabetic individuals (4). Diabetes and glucose intolerance are also more common in hypertensive than in normotensive patients (5). Hypertension is present in three of ten Jamaicans over 30 years of age (6). Recently, a 17.7% prevalence of diabetes mellitus in the Jamaicans aged over 15 years has been reported (7).

The choice of antihypertensive drug should be determined by the drug’s capacity to lower blood pressure and to protect the diabetic patient’s kidneys from ongoing injury and its side effects (8). Angiotensin converting enzyme (ACE) inhibitors such as captopril are used as first line therapy in people with type II diabetes and hypertension. They are effective in lowering blood pressure, usually well tolerated, and have an excellent metabolic profile (9). There is also increasing evidence that they offer protection against nephropathy through a specific effect on the renal microcirculation by reducing intraglomerular pressure (10). Bradykinin showed a dose-dependent increase in blood glucose levels in anesthetized rats (11).
infusion of bradykinin induces, via stimulation of B2 receptors, the release of nitric oxide (NO), an effect that was enhanced by captopril (12).

Previous studies have shown that captopril has a hypoglycemic effect with a capacity to lower blood glucose by increasing the muscular glucose disposal rate in hypertensive diabetic patients also treated with sulfonylureas and biguanidines (13). We postulate that captopril may not significantly inhibit the breakdown of bradykinin and hence increase the NO levels in sufficient amounts that would affect the postprandial blood glucose levels. The present study was designed to investigate the effect of captopril on systolic, diastolic and mean arterial blood pressures in normotensive dogs. The study also investigated the effect of captopril on blood glucose levels, plasma insulin and its effect on endogenous NO levels by means of measuring the plasma nitrate/nitrite levels taken as a biochemical index of NO in normoglycemic dogs.

MATERIALS AND METHODS

Normal adult mongrel dogs (10 male and 10 female) aged 2-3 years, mean weight 13.5 ± 0.5 kg, were obtained from the Preclinical Animal House of the Department of Basic Medical Sciences, University of the West Indies. The procedures that followed were in accordance with the university guidelines for animal studies. Dogs were fed a diet of standard Purina Laboratory Chow (Purina, St. Louis, MO, USA) and water administered ad libitum. The dogs used in the experiment were carefully selected based on having a mean arterial blood pressure in the range of 112-123 mm Hg, described as normotensive (14) and with fasting blood glucose levels of 4-6 mmol/L, i.e. normoglycemic. The dogs were divided into two groups, experimental (5 male and 5 female) and control (5 male and 5 female). Briefly, after an 18-h fast, dogs were anesthetized with sodium pentobarbital (30 mg/kg i.v.). The controls were administered 2 ml of deionized water (i.v.), while experimental dogs received 20 mg/kg of captopril (Sigma Chemicals, St. Louis, MO, USA) i.v.

Plasma glucose levels were determined by the glucose oxidase method (15), and absorbance was measured at 420 nm using a spectrophotometer (Spectronic Genesys). Deionized water or captopril was administered after a fasting blood sample of 0.5 ml (0' time point) had been taken from a vein in the forelimb. After a residence time of 30 minutes, a second fasting blood sample was taken at the 0-h time point. An oral glucose load of 1.75 g/kg body weight was given and blood samples were taken at half-hour time intervals for two and one-half hours after the oral administration of the glucose load. These blood samples were collected in ethylenediaminetetraacetic acid (EDTA) tubes and immediately placed on ice for subsequent biochemical analysis or were immediately analyzed for blood glucose, plasma insulin and plasma nitrate/nitrite concentrations. The plasma insulin concentration was measured by radioimmunoassay (Diagnostic Products Corporation, Los Angeles, CA, USA), and radioactivity was determined using a gamma counter (Abbott Auto Logic Gamma Counter).

NO formation was measured as nitrate and nitrite using Greiss reaction (16). Briefly, 50 µl of plasma were deproteinized by the addition of 100 µl of 35% sulfosalicylic acid. The treated samples were mixed by vortexing every 5 minutes and allowed to react for 30 minutes at room temperature. These were then centrifuged at 10,000 g for 15 minutes. Two hundred microliters (200 µl) of the supernatant were added to 4 ml of deionized water in a 1:20 dilution for analysis. The samples were then passed through a copper-cadmium column of an autoanalyzer (Technicon Instruments Corporation, New York, NY, USA) to reduce nitrate to nitrite. The resulting nitrite concentration was determined by the addition of Greiss reagent [0.1% sulfanilamide in 5% concentrated phosphoric acid and 0.1% N-(1-naphthyl)-ethylenediamine dihydrochloride] to form a purple azo dye and the nitrite was quantified using NaNO₂.

Mean arterial, diastolic and systolic blood pressure measurements were made in normotensive dogs. Captopril (20 mg/kg) was administered intravenously. Blood pressure recordings were performed at 10 minutes before the administration of 20 mg/kg of captopril or 2 ml of deionized water, and then at 10-minute intervals for 30 minutes after the administration of the drug. The systolic, diastolic and mean arterial blood pressures were also measured at the 2.5-h time point. The blood pressure measurements were performed using a clinical air plethysmograph/oscillometer (VascuMAP, model AP102V, Carolina Medical Electronics, NC, USA). All
studies were conducted in a temperature-controlled (23-25 °C), quiet environment. With the dogs in the supine position, forelimb mean arterial, systolic and diastolic blood pressures were measured using a standard-width air cuff (3-5 cm, according to the circumference of the forelimb). The blood pressure parameters were also measured at the 2.5-hour time point. This instrument was set to take mean arterial blood pressure to the nearest 0.1 mm Hg and waveform readings every one minute.

All results shown in the figures are expressed as mean ± SEM. Integrated area under the curve (iAUC) was calculated by subtracting the rectangle corresponding to the basal value from the total area under the curve (17). Analysis of the data was done using the Sigma Plot and Sigma Statistics software packages (Jandel Scientific). To evaluate the effect of captopril and water on the biochemical parameters, values for each group were compared by either paired Student’s test or two-way analysis of variance (ANOVA), followed by the Bonferroni multiple comparison test (18). The p values less than 0.05 were considered to indicate significance in all cases.

RESULTS

The dogs treated with 20 mg/kg of captopril and 2 ml of deionized water displayed normal glucose tolerance curves. The mean fasting blood glucose concentration in dogs treated with 20 mg/kg of captopril was 4.36 ± 0.40 mmol/L (Fig. 1). The peak postprandial blood glucose concentration was 6.90 ± 0.35 mmol/L at the 1-h time point. The blood glucose concentration decreased steadily to 4.70 ± 0.39 mmol/L at the 2.5-h time point. There were no significant differences between the blood glucose values at any of the time points between the two groups (p>0.05). The dogs administered water had a peak postprandial blood glucose concentration of 7.02 ± 0.35 mmol/L at the 1.0-h time point, which returned to the baseline value of 4.45 ± 0.29 mmol/L at the 2.5-h time point. The area under the glucose curve of dogs treated with captopril was 880.75 ± 65.68 mmol/L x 150 min compared with 976.54 ± 64.50 mmol/L x 150 min in dogs administered deionized water (p>0.05).

The fasting plasma insulin concentration in dogs treated with captopril was 9.40 ± 2.0 µIU/ml. The plasma insulin concentration increased to a maximum
of 42.30 ± 2.67 µIU/ml at the 0.5-h time point in response to glucose load and then decreased gradually to 10.12 ± 2.22 µIU/ml at the 2.5-h time point (Fig. 2). Similarly, the fasting plasma insulin concentration in the dogs administered water was 6.35 ± 0.50 µIU/ml. The plasma insulin concentration increased to a maximum value of 30.20 ± 2.25 µIU/ml at the 1.5-h time point in response to glucose load. The plasma insulin concentration then decreased to 12.50 ± 0.99 µIU/ml at the 2.5-h time point. Further evaluation of the data showed that there were statistically significant differences between the plasma insulin concentration of captopril-treated dogs at the 0.5-h time point as compared with the dogs administered water (42.30 ± 2.37 vs. 23.25 ± 1.64 µIU/ml; p<0.05). Similarly, the plasma insulin concentration in the captopril-treated dogs after 1.5-h was 21.50 ± 5.14 µIU/ml, compared with 30.20 ± 2.25 µIU/ml in the dogs administered water (p=0.12). The iAUC for dogs treated with 20 mg/kg of captopril was 5194.85 ± 76.31 mmol/L x 150 min as compared with 4667.56 ± 74.20 mmol/L x 150 min in the dogs treated with 2 ml of deionized water (p>0.05).

We analyzed plasma nitrate/nitrite concentrations in both captopril-treated and dogs administered water. The mean basal plasma nitrate/nitrite concentration in the fasting captopril-treated control dogs was 12.57 ± 0.4 µM. The plasma nitrate/nitrite concentration was fairly constant throughout the experimental period, with a peak concentration of 13.40 ± 1.01 µM at the 1.0-h time point, decreasing to 12.67 ± 0.75 µM at the 2.5-h time point (Fig. 3). A similar trend was observed in the dogs given deionized water.

The effects of 20 mg/kg of captopril and 2 ml of deionized water on the mean arterial blood pressure are shown in Fig. 4. Captopril elicited a blood pressure lowering response, with a decrease in the mean arterial pressure from 120.00 ± 6.00 mm Hg to 111.00 ± 4.00 mm Hg 10 minutes after the administration of the drug (-9.00 mm Hg). There was no significant decrease in the mean arterial blood pressure during the 10 to 20 minute intervals (-7.00 mm Hg). However, the mean arterial blood pressure decreased most sharply during the 20 to 30 minute interval from 101.00± 4.50 mm Hg to 90.00± 1.00 mm Hg. Deionized water had no effect on the mean arterial blood pressure at any time intervals during the experiment. There were significant differences in the
mean arterial blood pressure of captopril-treated and dogs administered deionized water at the 10-, 20- and 30-minute time points (p<0.05). The effect of captopril was more pronounced on systolic blood pressure (-32.00 ± 2.20 mm Hg; p<0.05) than on diastolic blood pressure (-19.00 ± 2.00 mm Hg; p<0.05) 30 minutes after drug administration. The systolic and diastolic blood pressures at the 2.5-h time point were 140.60 ± 9.00 mm Hg and 79.20 ± 4.50 mm Hg compared with the initial baseline values of 155.30 ± 2.50 mm Hg and 85.20 ± 3.00 mm Hg, respectively, before captopril administration. The mean arterial blood pressure at the 2.5-h time point was 98.30 ± 2.50 mm Hg.

DISCUSSION

The principal finding of the study was that normoglycemic dogs treated with captopril had normal glucose tolerance as indicated by decreased postprandial blood glucose levels to baseline values after oral glucose tolerance load. There was no significant difference in the postprandial blood glucose levels between the dogs treated with captopril and those administered deionized water, as reflected by the iAUC following oral glucose challenge. The normal glucose tolerance observed was accompanied by a fairly normal insulin response, although there was evidence of a sudden increase in the insulin level at the 0.5-h time point in response to oral glucose load. This could be due to the effect of captopril on the first-phase insulin release.

The finding of this study is similar to an earlier study by Winocour et al. (19), in which low-dose captopril therapy had no significant effect on blood glucose levels in hypertensive insulin-treated diabetics. This means that captopril did not have any effect on the insulin-mediated peripheral glucose disposal rate from muscular tissue (20), suggesting that captopril did not increase the risk of diabetes in these normoglycemic and normotensive dogs. Other investigators failed to demonstrate any effect of ACE inhibitors on glucose metabolism (21). They found that ACE inhibitors had no effect on glucose metabolism in type I diabetes (22) and chronic ACE inhibition on glucose tolerance and insulin sensitivity in hypertensive type 2 diabetes (23).

Captopril caused a significant reduction in systolic, diastolic and mean arterial blood pressures. Captopril is an orally effective inhibitor of the peptidyldepeptidasehydrolase. Captopril blocks the blood pressure response caused by the administration of angiotensin I (24). The mean arterial blood pressure-lowering capacity of captopril is related to a reduction in the peripheral arterial vascular resistance. The hypotensive response to captopril is accompanied by a decrease in plasma aldosterone and angiotensin II levels, and an increase in plasma renin activity (25).

Nitric oxide, now recognized as the most potent vasodilatory substance (26), plays a crucial role in the long-term regulation of systemic blood pressure and represents a physiological antagonist of angiotensin II (27). ACE inhibitors prevent both the generation of the potent vasoconstrictor angiotensin II and the degeneration of the vasodilator bradykinin, which promotes endothelial cell release of NO via endothelial B2 receptors. ACE inhibitors such as perindoprilate and captopril potentiate endothelium dependent relaxation to bradykinin whether given exogenously or formed locally in the blood vessel wall (28).

Bradykinin accumulation results in increased vascular permeability that might increase glucose and insulin delivery to the tissues (12). The results of the present study showed an increase of approximately 1 µM in the plasma nitrate/nitrite concentration, which is indicative of endogenous NO produced on captopril administration. This means that captopril did not significantly inhibit the bradykinin present in the proximity of the endothelial cells to effect significant NO production and thus affect the postprandial glucose levels. The normal glucose tolerance observed could be due to the effects of captopril on insulin-mediated glucose disposal that may be related to the drug’s hemodynamic effects (29). The vasodilatory action of captopril may increase the access of insulin and glucose to the skeletal muscle tissue, the main site of insulin-mediated removal of glucose (29).

In conclusion, we found that captopril reduced systolic, diastolic and mean arterial blood pressures in normoglycemic dogs, which was accompanied by normal glucose tolerance and normal insulin response to glucose load. There was no significant increase in plasma nitrate/nitrite either, suggesting that captopril facilitates glucose disposal via an NO-independent
mechanism that needs to be elucidated. The results of this study have contributed to current knowledge about the use of captopril.

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REFERENCES


