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Comparative antiplatelet effects of chlorthalidone and hydrochlorothiazide

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Abstract

Chlorthalidone (CTD) may be superior to hydrochlorothiazide (HCTZ) in the reduction of adverse cardiovascular events in hypertensive patients. The mechanism of the potential benefit of CTD could be related to antiplatelet effects. The objective of this study was to determine if CTD or HCTZ have antiplatelet effects. This study was a prospective, double-blind, randomized, three-way crossover comparison evaluating the antiplatelet effects of CTD, HCTZ, and aspirin (ASA) in healthy volunteers. The effects of these treatments on platelet activation and aggregation were assessed using a well-established method with five standard platelet agonists. Thirty-four patients completed the three-way crossover comparing pre- and post-treatment changes in platelet activation and aggregation studies. There were statistically significant antiplatelet effects with ASA but not with CTD or HCTZ. Hypokalemia occurred in 0 (0%), 10 (30%), and 6 (18%) of the ASA, CTD, and HCTZ patients, respectively. The results of our study suggest that the benefits of CTD and HCTZ in reducing adverse cardiovascular events in patients with hypertension is not a result of an antiplatelet effect. In our study, hypokalemia with CTD was more prevalent than that reported in a large outcome trial in patients with hypertension. The clinical relevance of this finding is uncertain.

KEYWORDS
antihypertensive therapy, clinical pharmacology, hypertension-general, pharmacologic (drug) therapy, potassium/hypertension

1 | INTRODUCTION

There is ongoing debate regarding the relative long-term benefits of chlorthalidone (CTD) and hydrochlorothiazide (HCTZ) in the management of hypertension. The 2017 American College of Cardiology/American Heart Association hypertension guidelines recommended CTD over HCTZ due to its longer half-life and proven reduction in major adverse cardiovascular events.[1] A retrospective analysis of the Multiple Risk Factor Intervention Trial (MRFIT) showed that CTD reduced cardiovascular events to a greater degree than hydrochlorothiazide (HCTZ) over a 7-year follow-up period.[2] A recent network meta-analysis found that CTD reduced the risk of heart failure and cardiovascular events by 23% and 21%, respectively, compared to HCTZ in trials with follow-up greater than 4 years.[3]

The mechanism of the potential benefit of CTD compared to HCTZ has not been established. CTD may have pleotropic effects unrelated to blood pressure lowering.[4] Laboratory studies suggest that CTD may have effects on platelet aggregation, gene transcription, angiogenesis, and vascular permeability not seen with other thiazide diuretics.[5,6] CTD inhibits carbonic anhydrase an enzyme associated with inhibition of platelet aggregation and promotion of angiogenesis.[6] These effects could explain the potentially greater effect of CTD in reducing the adverse vascular events observed in hypertensive patients. The objective of this investigation was to evaluate the comparative effects of CTD and HCTZ on platelet aggregation in healthy volunteers.

2 | METHODS

This study was a prospective, double-blind, randomized, three-way crossover comparison of CTD, HCTZ, and aspirin (ASA) in healthy volunteers. ASA was included in the study as a known control for inhibition of platelet aggregation. After obtaining informed consent, a history and physical examination and routine laboratory screening was performed to confirm that participants were healthy adults ≥ 19 years of age. Participants could not be taking any prescription medications, over-the-counter medications, or dietary or herbal supplements. Continuation of hormonal contraception was permitted. Use of any form of nicotine was an exclusion criterion. Use of non-steroidal anti-inflammatory drugs was not permitted during the 7-days prior to and throughout the duration of the study. All study procedures were approved by the Creighton University Institutional Review Board. Study participants were offered a stipend for time and travel.

A Williams design was used to randomize participants to one of six possible treatment sequences of treatment periods 1, 2, and 3, respectively: ABC, BCA, CAB, ACB, BAC, and CBA (A: HCTZ 25 mg daily, B: CTD 12.5 mg daily, and C: ASA 81 mg daily). This design ensured that carryover effects of treatment would not impact study outcomes.[7] Each study period was 2 weeks in duration with a 2-week washout between study periods. Blinding of study medications was accomplished using encapsulation. Randomization was performed using a computer-generated randomization list.

At the start of each treatment period, participants received blinded study medication and had baseline platelet reactivity measurements performed. Participants returned 1 week later to have a basic metabolic panel measured. At the end of each 2-week treatment period, patients returned for repeat platelet reactivity testing. Each study period was followed by a 2-week washout prior to starting the next treatment period. If the serum potassium level after 1-week of treatment was between 3.0 and 3.7 mEq/dl, patients were given potassium 20 mEq per day for the remainder of that treatment period. If the potassium level was < 3.0 mEq/dl, the patient was removed from the study.

Blood samples for platelet aggregation were collected in buffered 3.2% sodium citrate tubes with a 9:1 ratio of blood to anticoagulant. All samples were analyzed within 4 h of collection on a Chrono-log Lumi-Aggregometer 700(Chrono-Log Corporation, Havertown, PA, USA). The instrument uses an impedance method of aggregation measurement. The impedance method allows the study of platelet agonist responses in whole blood and minimizes platelet manipulation prior to assay. The change in impedance is directly related to platelet aggregation on the instrument electrodes and is displayed as a function of time. The peak impedance was measured in ohms. ATP release was measured by a luminescence technique (recombinant luciferin-luciferase) simultaneously with the impedance-measured aggregation response. Extra-cellular ATP was measured via photomultiplier tube filtered to detect the wavelength of light created by the CHRONO-LUME reagent. Measured light was then converted to nmols of ATP via calibration curve. All aggregation and secretion measurements were performed using the Chrono-log AGGRO/LINK8 software.

Five agonists were utilized to measure aggregation and secretion responses. The agonists included collagen at 1 ug/ml (low dose) and 5 ug/ml (high dose), ADP at 20 uM, arachidonic acid at .5 nM, thrombin at 1 U/ml (secretion only) and ristocetin at 1 mg/ml (high dose) and .25 mg/ml (low dose) (aggregation only). Reference intervals for aggregation and secretion responses were established at the time of instrument validation using control specimens.

Platelet reactivity were summarized by their respective means ± standard deviations or percentages as appropriate. For this crossover trial design, the presence of carryover effects was investigated by examining the washout periods. A multivariable linear mixed effects model with identity link was utilized and included a patient random effect and the fixed effects of washout period and drug order. Mean values for each outcome measurement are presented for each washout period to compare to normal reference values. Aspirin was set as a comparative reference for the investigation of CTD and HCTZ. Model residuals were examined for normality and equal variance. After adjusting for the effect of period, the comparisons of CTD and HCTZ to aspirin incorporated a Dunnett-Hsu adjustment for multiplicity. Imputation for missing data was not employed. Data analysis was conducted using SAS v. 9.4(Cary, NC, USA) with a $p < .05$ indicating statistical significance. The primary endpoint was the degree of platelet aggregation as determined by electrical impedance due to CTD, HCTZ, and aspirin in the presence of the agonist collagen, ADP, arachidonic acid, thrombin and ristocetin.

3 | RESULTS

Forty healthy patients provided informed consent and were randomized into the study. Six patients withdrew consent and failed to complete the three-way crossover. Three patients were withdrawn from the study for noncompliance while three patients were withdrawn for adverse effects prior to completion of the three-way crossover. One patient experienced an increase in serum creatinine on HCTZ, one patient complained of lightheadedness and nausea on CTD, and one patient complained of blurred vision, lethargy, and “swollen tonsils” on CTD. Thirty-four participants (85%) completed all study visits in the three-way crossover and were included in the final comparative platelet aggregation analysis (Table 1).

TABLE 1 Baseline demographics of study participants

Sex, *n* (%)	Male	13 (38.2)
	Female	21 (61.8)
Age (years), mean (SD)		23.7 (7.1)
Weight (kg), mean (SD)		71.7 (13.5)
Height (cm), mean (SD)		154.9 (9.8)
Race, *n* (%)	White	25 (73.5)
	Asian	5 (14.7)
	Black	3 (8.8)
	Multiracial	1 (2.9)

The results of the platelet aggregation studies are summarized in Table 2. Only ASA demonstrated significant differences in the measures of platelet aggregation after 2 weeks of treatment. Seven of the 11 of platelet aggregation and secretion measurements were significantly affected by ASA. None of 11 measurements were affected by HCTZ and one of 11 measurements were affected by CTD. When the pre- and post-treatment changes in platelet function were compared for CTD and HCTZ, there were no statistically significant differences between them for any measure. ASA produced statistically significant greater effects in seven of 11 measures of platelet function when compared to CTD and to HCTZ.

Mean serum potassium concentrations were significantly lower during treatment with HCTZ (3.72 ± .27 mEq/L; $p < .001$) and CTD (3.64 ± .36 mEq/L; $p < .001$) compared to baseline (4.03 ± .22 mEq/L) [Table 3]. Treatment with ASA (4.04 ± .21) had no effect on serum potassium concentrations. The magnitude of the change in serum potassium was not statistically different between HCTZ and CTD. The proportions of patients experiencing a serum potassium < 3.7 mEq/L and a serum potassium < 3.5 mEq/L at any time during baseline and with each active treatment is summarized in Table 3. HCTZ and CTD were associated with statistically significant increased proportions of patients with hypokalemia and serum potassium levels triggering potassium supplementation compared to ASA. The proportion of patients developing hypokalemia and serum potassium levels triggering potassium supplementation were not different between HCTZ and CTD ($p = .44$). No patient was removed from the study as a result of a serum potassium < 3.0 mEq/L.

4 | DISCUSSION

Data from several large clinical trials and meta-analyses suggests that despite similar reductions in blood pressure CTD may have relatively greater cardiovascular benefits compared to HCTZ in the treatment of hypertension.[3,8–10] The ongoing Diuretic Comparison Project (DCP) being conducted by the Department of Veterans Affairs may offer some clarity on this issue.[11] It will be the first large-scale, prospective, randomized, direct comparison of CTD and HCTZ with a primary outcome of cardiovascular events. Until the results of this trial are known, uncertainty regarding the relative benefits of CTD and HCTZ on major adverse cardiovascular events in hypertensive patients will persist.

Our trial investigated the effects CTD and HCTZ on platelet activation and aggregation which has been suggested to be a potential reason for the cardiovascular benefit of CTD. Woodman and coworkers, conducted a number of in vitro comparisons of the effects of CTD and bendroflumethiazide on platelet carbonic anhydrase activity, catecholamine-induced platelet aggregation, vascular permeability, and endothelial cell angiogenesis.[6] Compared to bendroflumethiazide, CTD produced a significantly greater inhibition of carbonic anhydrase activity, was more effective in preventing epinephrine-mediated platelet aggregation, and increased vascular smooth muscle cell angiogenesis. These findings suggested that thiazide diuretics vary in their pleiotropic effects on platelets and in the vasculature.

Our study did not find a significant effect of either CTD or HCTZ on measures of platelet activation or aggregation while ASA did produce a significant effect on platelet aggregation. Why the antiplatelet effects of CTD observed in the in vitro experiments of Woodman and coworkers, were not seen in our study is uncertain.[6] Translation of pharmacologic findings observed in vitro models to the clinical setting is challenging. The in vitro data identifying an antiplatelet effect with CTD used bendroflumethiazide as a control. Neither of these thiazide diuretics has previously been identified as having effects on platelet function. In addition, only epinephrine was used in this study as an agonist to stimulate platelet activation and aggregation. Our study assessed the impact of CTD and HCTZ on platelet activation and aggregation using state-of-the-art laboratory methods including five different platelet agonists and a well-known antiplatelet agent (ASA) as a control.[12] An older study of 80 middle-aged mildly hypertensive patients found no effect of HCTZ 25 mg taken daily for 1 month on platelet aggregation in response to ADP or arachidonic acid.[13] The available evidence suggests that neither CTD or HCTZ is associated with antiplatelet effects in humans.

CTD was associated with a 30% prevalence of hypokalemia in our study which is higher than that reported in the ALLHAT trial.[14] The ALLHAT trial compared CTD against lisinopril and amlodipine as first-line therapies in the primary prevention of myocardial infarction in 42000 patients with hypertension. CTD was associated with a 13% incidence of hypokalemia.[15] Hypokalemia occurred in 1% and 2% of patients treated with lisinopril and amlodipine, respectively. In the overall ALLHAT study population, hypokalemia was associated with a statistically significant increase in cardiac (18%) and non-cardiac (23%) death. When analyzed by treatment group, however, hypokalemia was not associated with an increased risk of death in CTD patients.[15] It was suggested that the hypokalemia and an excess risk of death among patients treated with the other antihypertensive drugs occurred due to cancer and other conditions associated with excess potassium losses.[14,15] In an observational assessment of 1.4 million hypertensive patients in the Large-Scale Evidence Generation and Evaluation in a Network of Databases (LEGEND) study, CTD was associated with an excess rate of hypokalemia compared to HCTZ.[16] However, the composite endpoint of adverse cardiovascular events was not significantly

TABLE 2 Effect of treatment on measures of platelet aggregation

	Collagen low aggregation	Collagen low secretion	Collagen high aggregation	Collagen high secretion	ADP aggregation	ADP secretion	AA aggregation	AA secretion	Thrombin secretion	Ristocetin low aggregation	Ristocetin high aggregation
ASA baseline, mean (SD)	17.97 (3.24)	1.00 (.46)	19.65 (6.20)	1.42 (.68)	16.44 (6.39)	1.03 (.72)	13.09 (5.55)	1.24 (.90)	1.52 (.60)	19.32 (1.66)	.029 (.17)
ASA post, mean (SD)	12.44 (5.96)	.52 (.27)	19.59 (6.13)	1.12 (.39)	16.79 (8.38)	.44 (.41)	2.71 (6.23)	.24 (.42)	1.37 (.63)	8.59 (5.63)	.103 (.30)
ASA Pre-post difference (p)	<.001	<.001	.96	.00	.80	<.001	<.001	<.001	.31	<.001	.231
CTD baseline, mean (SD)	19.59 (5.24)	1.24 (2.11)	20.15 (6.95)	1.25 (.49)	17.47 (6.33)	.75 (.40)	14.32 (6.79)	.98 (.61)	1.30 (.61)	19.24 (7.66)	.029 (.17)
CTD post, mean (SD)	17.62 (3.90)	1.03 (.43)	21.47 (5.66)	1.33 (.48)	15.97 (5.70)	.77 (.51)	14.94 (5.93)	1.16 (.69)	1.40 (.62)	19.12 (8.05)	.088 (.379)
CTD pre-post difference (p)	.03	.58	.22	.41	.26	.84	.51	.19	.43	.95	.422
HCTZ baseline, mean (SD)	18.26 (4.28)	1.05 (.40)	20.53 (6.95)	1.43 (.47)	17.32 (6.27)	.85 (.42)	13.35 (7.07)	.95 (.53)	1.53 (.66)	17.74 (8.40)	.059 (.34)
HCTZ post, mean (SD)	17.62 (5.89)	.96 (.39)	19.82 (6.08)	1.36 (.51)	15.53 (5.98)	.77 (.51)	12.62 (6.78)	.99 (.50)	1.50 (.60)	16.71 (6.86)	.000 (.00)
HCTZ pre-post difference (p)	.56	.36	.55	.57	.16	.39	.56	.70	.84	.52	.325

TABLE 3 Serum potassium levels with treatment

	Mean ± SD	Hypokalemia (< 3.5 mEq/L)	Potassium supplementation (≤ 3.7 mEq/L)
Baseline	4.03 ± .22		
ASA	4.04 ± .21	0	2 (6%)
CTD	3.64 ± .36*	10 (30%)	18 (53%)
HCTZ	3.72 ± .27*	6 (18%)	17 (50%)

*$p < .001$ for baseline to on-treatment; difference in mean CTD versus HCTZ, p = .287.
Hypokalemia (K < 3.5): CTD versus ASA $p < .001$; HCTZ versus ASA $p = .025$; CTD versus HCTZ $p = .392$.
K+ supplementation: ASA versus CTD $p < .001$; HCTZ versus ASA $p = .001$; CTD versus HCTZ $p = 1.00$.

different between CTD and HCTZ. The clinical impact of hypokalemia among patients receiving CTD and HCTZ remains uncertain.

There are several potential limitations that may have impacted the findings in our study. Given the small sample size, it is possible that a type II error limited our ability to detect an antiplatelet effect with CTD or HCTZ. This is unlikely, however, given the consistent effect of ASA on measures of platelet aggregation and the consistent lack of effect of both CTD and HCTZ on measures of platelet aggregation. The use of a crossover design allowed for each participant to serve as their own control which would minimize the potential failure to detect a difference if one existed. In addition, in vitro tests of platelet function vary widely in terms of methodology, reproducibility, and correlation with clinical outcomes. The inclusion of ASA as the active control allowed for confirmation that the laboratory methods used for platelet aggregation testing in our study was valid. The use of multiple agonists in the testing protocol also allowed for robust evaluation of potential differential effects on platelet function. The administration of potassium supplements in our study to patients with a serum potassium ≤ 3.7 mEq/L may have diminished platelet reactivity and altered our ability to detect an antiplatelet effect with CTD or HCTZ. Kimura and coworkers, observed a reduction in platelet aggregation in response to ADP in healthy patients supplemented with relatively high doses (60 mEq per 70 kg body weight) of potassium chloride in an experimental study.[17] The patients in this experimental study were not receiving diuretics and had normal serum potassium levels. The effects of CTD or HCTZ on platelet function in our study were not likely to have been impacted by potassium supplementation as our results were not different in patients receiving and not receiving potassium supplementation.

Current hypertension guidelines recommend CTD over other thiazide-like diuretics.[1] Whether CTD produces a cardiovascular benefit compared to HCTZ in the treatment of hypertension is still a topic of active debate. The completion of an adequately powered head-to-head outcome trial is needed to confirm such a benefit. While previous in vitro work has suggested that effects on platelet activation and aggregation may be a potential mechanism to explain differences in outcomes between CTD and HCTZ, the results of the present study did not find an effect of either drug on platelet activation or aggregation.

AUTHOR CONTRIBUTION

The authors confirm contribution to the paper as follows: Study conception and design: Drs. Khalid Bashir, Tammy Burns, Samuel Pirruccello, Sarah Aurit, Daniel Hilleman. Data collection: Drs. Khalid Bashir, Tammy Burns, Samuel Pirruccello. Analysis and interpretation of results: Drs. Khalid Bashir, Tammy Burns, Samuel Pirruccello, Sarah Aurit, Daniel Hilleman. All authors reviewed the results and approved the final version of the manuscript.

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CONFLICTS OF INTEREST

None of the authors have conflicts of interest.

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