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Increased Autonomic Dysfunction in Subjects Treated with Alpha-Methyldopa

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Abstract

Methyldopa is a catecholamine used as an antihypertensive agent. The purpose of this study was to evaluate the effect of a week treatment of a bi-daily dosage of α-methyldopa on the sympathetic nervous system of healthy volunteers as measured by four different techniques; (QT interval, heart rate variability (HRV), skin conductance and salivary cortisol). All volunteers received either 250mg α-methyldopa orally or a placebo tablet in a randomized, double blind, placebo controlled study design. The correlation between the following techniques was also evaluated: Skin conductance, QT interval on ECG and HRV measured with Viport apparatus. A salivary sample was collected to evaluate the effect of α-methyldopa on salivary cortisol using an ELISA kit. After a week treatment, the systolic blood pressure and heart rate were significantly decreased. In both the placebo and the treatment groups the cardio stress index (CSI) decreased. Within the groups only the treatment group had a statistically significant result. The study revealed a decreasing effect on blood pressure, heart rate, CSI, standard deviation of the RR interval (RRSD) and QTc, whilst an increasing effect on QRS complex, HRV, QT interval, skin conductance and salivary cortisol.

Keywords: Methyldopa, autonomic dysfunction, sympathetic nervous system, QT interval, heart rate variability

1. Introduction

Methyldopa (α-methyl-3, 4-dihydroxy-L-phenylalanine) is an analog of DOPA (3,4-hydroxyphenylanine). (Norouzi et al.2009). It was discovered over 50 years ago, with its blood pressure lowering effects being noted in 1959 (Stein and Bronner1955) The Joint National Committee (JNC) recommended methyldopa as add on therapy for hypertension after diuretics in 1977 (JNC 1977).

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Methyldopa is no longer used frequently due to its side effects, but it is still used, especially in developing countries because of its low cost. It is mostly indicated for hypertension, in particular for the management of pregnancy-induced hypertension, as it is relatively safe in pregnancy compared to other antihypertensive drugs. Methyldopa as treatment for severe dyskinesias is also recognised (Toxnet 2012). According to the prescribing information of methyldopa, its exact mechanism of pharmacological action is not known (HY-PO-TONE package insert). Various mechanisms of action have been hypothesized in previous studies (Halushka and Keiser 1974). The antihypertensive effect most commonly accepted is the metabolism of methyldopa to α-methyl norepinephrine (NE). Methyldopa is metabolized by L-aromatic amino acid carboxylase in adrenergic neurons to α-methyl dopamine which is then converted to α-methyl NE. Thus when adrenergic neurons discharge their neurotransmitters, α-methyl NE is released instead of NE (Brunton et al. 2006). Alpha-methyl NE is as potent a vasoconstrictor as NE, thus its substitution for NE in peripheral adrenergic neurosecretory vesicles does not alter the vasoconstrictor response to peripheral adrenergic neurotransmission. Rather adrenergic neuronal outflow is inhibited due to the effect of α-methyl NE in the central nervous system. According to the literature, methyl NE probably acts as an agonist at presynaptic auto-inhibitory α_2 adrenergic receptors in the brainstem, attenuating NE release and thereby reducing the output of vasoconstrictor adrenergic signals to the peripheral sympathetic nervous system (Brunton et al. 2006).

The most common side effect of Methyldopa is drowsiness. Some other frequent adverse events include depression, psychic effects, impaired mental acuity, nightmares, nausea, dryness of the mouth, nasal stuffiness, weakness, dizziness, light-headedness, headache, oedema and disorders of sexual function (HY-PO-TONE package insert). Caution should be taken when methyldopa is administered to people with impaired kidney or liver function or with a history of liver disease or mental depression. As expected, methyldopa is contra-indicated in anyone sensitive to the compound. It is also reported that porphyria is aggravated by methyldopa?(HY-PO-TONE package insert). The peak plasma concentration is achieved after 2 to 3 hours, whereas the maximum fall in arterial pressure is only observed after 6 to 8 hours. Thus the plasma level of methyldopa does not correlate with its clinical effect. (Ellenhorn and Barceloux 1988)The initial treatment dosage is 250mg orally, 2 to 3 times daily. Thereafter the maintenance dosage is between 500 mg and 2g orally divided in 2 to 4 doses. No advantage is obtained at doses larger than 3 g daily.

A gradual hypotensive response occurs in most patients in 12 to 24 hours (De Vito et al. 2002). Methyldopa produces a number of side effects due to alpha-2-adrenergic agonist effects. The present work was thus formulated to investigate the effects of methyldopa. In particular, the effects on the sympathetic nervous system were evaluated after the administration of a bi-weekly dose of methyldopa in healthy volunteers. Furthermore the effects on the sympathetic nervous system was assessed using four different techniques viz., QT interval, heart rate variability (HRV), skin conductance and salivary cortisol.

The role of α-2 agonists to treat attention deficit hyperactivity disorder (ADHD) commenced with clonidine since it has potent presynaptic actions in decreasing NE release and inhibiting the firing of NE locus ceruleus neurons. (Arnsten 2010). Emerging research shows that guanfacine, a α-2A agonist has multiple advantages: (1) it is better tolerated than other stimulant medications, (2) it can reverse stress-induced prefrontal cortex deficits in animals, (3) it is more helpful in patients that are anxious or those with post-traumatic stress disorder, (4) it is safer treatment for children tentatively diagnosed with ADHD especially those with emerging bipolar disorder or schizophrenia, (5) it may help patients with conduct disorders who have underactive orbital prefrontal cortex function, (6) it plays a role on overcoming harmful emotional habits; such as drug addiction, (7) it reduces aberrant behaviours in children with autism spectrum disorders, and (8) it assists patients cognitive function when recovering from parietal cortex strokes (Arnsten 2010). α-methyl nor epinephrine, stimulates centrally located α-adrenergic receptors in similar fashion to that of clonidine and guanfacine. (Van Zwieten et al. 1984). This study shows that methyldopa causes changes in the sympathetic nervous system as reflected by changes in the blood pressure, heart rate, ECG variables and heart rate variability.

2. Materials and Methods

In this randomised double-blind placebo controlled parallel trial, healthy normotensive volunteers who had given written informed consent, received either 250mg alpha-methyldopa or placebo orally, twice a day for one week. Volunteers were randomly divided into 2 groups. Participants consisted of healthy male and female volunteers using reliable contraception, between 18 and 30 years old. Only volunteers who were willing to sign the informed consent form were enrolled. Those with serious diseases were excluded.

Twenty-two participants were screened and enrolled in the study. Two participants withdrew from the study and did not complete the final visit of the study. Participants were reviewed twice. At visit 1, baseline, study related procedures and data collection were done. At visit 2, upon completion of one week treatment, post-treatment data was collected as shown in Table 2. Volunteers were monitored for study variables in the sitting position. This clinical study protocol has been approved by the University of the Pretoria Research Ethics Committee (Protocol S198/2010). The study and informed consent were conducted in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines. Participants were monitored by inclusion and exclusion criteria:

2.1. Parameters Measured

2.1.1. Blood pressure (BP) and heart rate (HR)

BP and heart rate were measured using a Microlife blood pressure monitor (Model: BP3BT0-A). Arm measurements were done. Subjects were measured in sitting position after a5 minutes of rest period

2.1.2. Skin Conductance

Biofeedback is a painless and non-invasive procedure to measure the skin conductance. Sensors/ electrodes were attached to the index and middle finger after the area had been wiped with an alcohol swab. The electrodes were connected to the ProComp Infiniti Biofeedback apparatus which was also connected to a computer. Using the BioGraph Infiniti Software 3.0 program the skin conductance could be measured while subjects were in a sitting position. A reading of 5 minutes was taken for each subject at the baseline and at the final visit.

Table 2: Descriptive Statistics of Variables at Baseline and Final Visit

Measurements	Placebo group						Treatment group						Expected direction of change
	Baseline			Final			Baseline			Final			
	Median	Mean	SD	Median	Mean	SD	Median	Mean	SD	Median	Mean	SD	
Vitals													
BP: systolic (mmHg)	119.0	124.9	13.51	124.5	123.2	11.3	128.5	125.4	14.8	120.0	117	9.02	↓
BP: diastolic (mmHg)	78.5	79.3	12.83	80.5	80.1	5.74	83.5	80.1	8.6	78.0	78.8	4.69	↓
Pulse (bpm)	75	74.9	14.45	79.5	80.1	12.29	74.0	78	16.59	72.0	71.2	11.36	↓
Viport													
Pulse: Viport (bpm)	74.5	71.8	12.33	81.0	81.4	12.68	74.5	77	11.03	71.5	74.4	13.2	↓
CSI	17.0	25.3	19.79	16.5	21.7	13.47	21.5	30.4	24.53	16.5	19.4	12.32	↓
QRS (ms)	85.0	81.8	15.1	74.0	78.7	13.21	74.5	72.5	13.08	67.0	72.7	15.12	↑
RRSD (ms)	62.5	58.2	12.58	40.5	41.7	10.7	54.0	70	42.1	48.5	54	24.56	↑
ECG													
QT interval (ms)	374.5	359.5	28.52	361.5	358.2	20.89	371.0	376.7	29.5	383.0	385.6	37.43	↓
Pulse: ECG (bpm)	69.0	72.1	17.16	71.5	72.9	10.95	68.5	72.4	12.66	63.5	64.9	11.61	↓
QTc (ms)	391.5	400.4	25.13	396.5	393.6	25.37	411.5	412.7	22.59	402.0	398	21.18	↓
Biofeedback apparatus													
Skin conductance	2.84	3.01	0.92	2.75	3.21	1.95	2.64	3.03	1.14	2.74	3.3	2.11	↓
ELISA													
Salivary cortisol [ng ml-1]	1.19	3.24	5.28	0.08	1.44	3.28	0.13	3.00	5.99	0.15	0.12	0.13	↓

2.1.3. QT interval

12 lead Electrocardiogram (ECG) was used. After 5min in the supine position the ECG was taken with the Edan SE-1200 ECG machine.

2.1.4. HRV

2.1.5. The Viport is a small electronic handheld device that was used to measure HRV via Cardio Stress Index (CSI). The data was collected on the basis of a digital multi-channel ECG system while subjects were in the sitting position. Before the device was used, the electrodes were prepared by adding electrode gel to each electrode to make them moist. The Viport was positioned on the subjects' bare chest three fingers width below the left clavicle. All the electrodes were in direct contact with the skin during the entire measurement.

After approximately 2 minutes of data recording the Viport computed an individual electrocardioportrait (ECP) on the integrated colour display from the recorded signal, as well as the following values: (i) CSI (range 0-100), (ii) Heart rate (beats min-1), (iii) Rhythm (rhythmic yes/no), (iv) QRS duration (the length of ventricle activity in your heart) and (v) RRSD (ms) (the standard deviation of the interval between the individual heart beats). The RS length, HR, rhythm and RRSD of the ECG is used to calculate the CSI. Via algorithms these parameters are transformed to give CSI which then can provide information about HRV. The range of measurement is between 0 and 100. A normal CSI is considered between 0 and 20 and corresponds to a high variability. Above 20 indicates reduced variability. A decrease in RRSD-values indicates a reduced HRV which means an increased CSI. Salivary cortisol

Saliva is a convenient, non-invasive method of determining hormonal concentrations (Thomas et al. 2009). Studies have proven the validity of salivary assays in the clinical and non-clinical field (Bahar et al. 2007).An Enzyme-linked immunosorbent assay (ELISA) for the in-vitro diagnostic quantitative determination of free cortisol in human saliva was done. The Cortisol ELISA kit (RE52611) from IBL International that was used has an analytical sensitivity (limit of detection) of up to 0.05 ng mL-1 and a functional sensitivity of up to 0.30 ng ml-1. Salivary cortisol levels peak in the first 90 minutes after awakening. To control for diurnal variation in cortisol concentration within participants each visit was conducted at approximately the same time of day. No food, drinks, gum or brushing of teeth was allowed 30min before sampling. Saliva was collected in an eppendorf tube. A minimum of 0.5ml liquid was collected. Samples were frozen at –20°C prior to laboratory testing. The protocol of the ELISA kit (RE52611) from IBL International was followed for samples collected. According to a study done by Westermann *et al.*, the saliva cortisol range measured in ng mL-1 in healthy subjects is between 2.0 – 14.1 before midday and between 0.4 – 4.1 after midday (Westermann et al. 2004). The frozen samples were brought to room temperature on the day of the assay. They were carefully mixed and centrifuged for 10min at 2000xg. Only the clear colourless supernatant was used. Before the assay was started a pre-test was done. One randomly selected sample was used to make a 1:10 and 1:100 dilutions. These dilutions plus the undiluted sample were used for the complete assay procedure. Because the undiluted specimen was found to contain more than the highest standard, all the saliva samples were diluted to 1:10 by adding 10µl saliva to 90µl Standard 0.

All the samples were used for the following assay procedure: 100µl of each standard, control and samples were dispensed into appropriate wells. Only 9 out of the 10 subjects' samples were used for the analysis with the ELISA kit due to the limited wells available on the plate. The 9 samples were randomly selected from the available 10 samples. 200µl of the enzyme conjugate was pipetted into each well and mixed thoroughly for 10 seconds. The microtiter plate was incubated for 60min at room temperature on a shaker at 300rpm. The contents were briskly removed from the plate. The wells were rinsed 5 times with the diluted wash solution using 400µl per well each time around. 200µl of the substrate solution was added to each well. The plate was incubated for 30min at room temperature. The enzymatic reaction was stopped by adding 100µl of the stop solution to each well. The absorbance was measured at 450nm within 10 min after adding the stop solution. A standard curve was generated using the controls.

2.2. Statistical Analysis

One-sided T-tests for paired observations were used to assess whether alpha methyldopa causes a decrease in sympathetic activity in each of the two groups from baseline to the final measurement. Independent samples T-tests were conducted to evaluate the difference in change from baseline to the final measurements between the control and the treatment groups. Because the assumptions of the T-tests were violated and the sample was too small to rely on the central limit theorem, analogous nonparametric tests (Wilcoxon signed-rank and Mann-Whitney) were performed instead. The exact significance values are reported. The level of significance is specified as $\alpha = 0.05$; in view of the small sample size, p-values larger than 0.05 but smaller than 0.10 are also reported as moderate evidence against the null hypothesis (Albright et al. 2010).

3. Results

In total, 20 subjects participated and completed the clinical trial. The 20 subjects were randomly divided into the placebo or the treatment group, 10 in the placebo group and 10 in the treatment group. The average age of the volunteers was 23.55 years, with a range of 18 to 29 years (Table 1). Only 30% of the volunteers were female and the remaining 70% male. Although this is not a representation of the South African population, the volunteers were randomly selected.

Table 1: Participant Demographics and Baseline Characteristics

Baseline characteristics	Placebo Group	Treatment group
Age		
Mean	24.8	22.3
Range	21-29	18-29
Sex		
Male	8	6
Female	2	4

Table 2 displays the descriptive statistics of the two groups for the variables of interest at baseline and the final visit. The statistical results (p-values of the Wilcoxon signed rank tests for within subject evaluation and the Mann-Whitney tests for the change between the two groups) are presented in Table 3.

Table 3: Statistical Results

	Wilcoxon rank sum (Within subjects) Baseline	Final	MWU (Between subjects)
Vitals			
BP - systolic (mmHg)	0.4	0.033*	0.124
BP - diastolic (mmHg)	0.297	0.318	0.177
Pulse (bpm)	0.023*	0.052	0.010*
Viport			
Pulse (Viport)	0.006*	0.087	0.003*
CSI	0.271	0.004*	0.053
QRS (ms)	0.238	0.297	0.218
RRSD (ms)	0.003*	0.318	0.038*
ECG			
QT interval (ms)	0.101	0.043*	0.026*
QTc (ms)	0.361	0.012*	0.197
Pulse (ECG)	0.111	0.013*	0.038*
Biofeedback apparatus			
Skin conductance	0.48	0.439	0.398
ELISA			
Salivary cortisol [ngml-1]	0.26	0.06	0.273

2.1. BP and HR

With regards to BP, the placebo group showed little change within the group when comparing the baseline visit to the final visit. Though no change was expected in the placebo group, statistically significant increases in the HR were observed when measured with the BP monitor (p=0.023) and viport (p=0.006) after a week of Methyldopa treatment, BP and HR decreased in the treatment group. The systolic BP and three HR measurements [measured with BP monitor, (p=0.052, moderate evidence); viport (p=0.087, moderate evidence) and ECG apparatus (p=0.016, strong evidence)] decreased significantly as seen in Table 3. The diastolic BP also decreased, however it was not significant. Comparing the placebo group with the treatment group there was statistical significance for all the heart rates measured with the different apparatus, indicating strong evidence that Methyldopa has a decreasing effect on the HR. Although there was a decrease in the treatment group for both systolic and diastolic BP, compared to the placebo group, the difference was not significant. Since methyldopa is an anti-hypertensive agent, one would have expected a decrease in BP. A reason for this occurrence may be the type of population that was used. All the subjects were young (18-30 years), healthy and normotensive. Although an anti-hypertensive agent was administered, the body's homeostatic regulation may have maintained pre-treatment BP.

3.2. Cardio Stress Index (CSI) and Heart rate Variability (HRV)

The Cardio Stress Index (CSI) is a converted measure of HRV. Various parameters of HRV were measured through frequency domains with the viport apparatus and are represented as a percentage namely the CSI. A normal CSI is considered between 0 and 20 and corresponds to normal HRV. Methyldopa suppresses the sympathetic nervous system causing HR to decrease but inversely increasing HRV. As seen in Table 2, in both the placebo and the treatment groups, the CSI decreased. Within the groups only the treatment group had a statistically significant result, p=0.004 as shown in Table 3. The decrease in CSI correlated with higher HRV. Thus Methyldopa has a moderate increasing effect on HRV. Interestingly, the Mann-Whitney test revealed a p=0.053 between the 2 groups which is suggestive of strong evidence.

3.3. QRS Complex and RRSD

A study done by Folino (Folino et al.1995) indicated that an increased sympathetic stimulation causes a decrease in QRS complex duration. Methyldopa decreases sympathetic output, causing the QRS complex to possibly increase. A normal QRS complex is considered between 60 and 100 milliseconds. Within each of the two groups and between the two groups the QRS complex did not change significantly. Although the placebo group showed a slight decrease, the treatment group had a slight increase. The average baseline and final visit QRS complex values for both the placebo group and the treatment group were within the normal range of 60 and 100 milliseconds. The RRSD is the standard deviation of the interval between each heartbeat. A reduction in RRSD reflects a shift toward more sympathetic dominance (Schmidt et al., 2010). A higher RRSD represents a higher discrepancy between the R-R intervals on an ECG. A low RRSD is accompanied by a low HRV which will lead to a high CSI. Very high RRSD's may be an indication of an arrhythmia. Both RRSD variables decreased within their specific group, although only the placebo group showed a significant decrease.

3.4. QTc

A corrected QT (QTc) interval is used instead of the normal QT interval since QT varies with heart rate; therefore QTc is adjusted for effect of the heart rate on the QT interval. A normal QTc interval range is considered to be between 0.35–0.44 seconds. Within the groups both showed a decrease; nevertheless only the treatment group had a significant decrease. A longer HR-QTc interval represents predominance of sympathetic activity within autonomic balance (Nagaya et al., 2010). We can thus assume that sympathetic activity was suppressed with Methyldopa. However, when comparing the groups with one another, there was no significant difference between the two groupsfor the QTc. The p-value for the QT interval is significant though as shown in Table 3.

3.5. Skin Conductance

With the suppression of the sympathetic nervous system the skin conductivity is lowered due to the lack of sweat in the eccrine glands. A slight increase in skin conductance was observed in both the placebo and treatment group comparing the baseline with the final measurement.

No significant result was obtained when comparing the difference between the placebo and the treatment group.

3.6. Salivary Cortisol

Only 9 out of the 10 subjects' samples were used for the analysis with the ELISA kit due to the limited wells available on the plate. The 9 samples were randomly selected from the available 10 samples. To calculate the cortisol concentrations of the salivary samples a standard curve was generated using the absorbance values of the standard controls that were supplied. The average absorbance value of each subject's salivary sample was used to calculate the cortisol concentration. These average absorbance values were substituted in the equation ($y = -0.2739x + 1.3872$) of the standard curve. Where 'y' represents the absorbance value of the saliva, and 'x' is the cortisol concentration that was calculated. A study done by Campisi (Campisi et al., 2012) indicated that salivary cortisol levels increase after sympathetic arousal and even stays elevated after 30 minutes when blood pressure and heart rate returned to their resting values. Both the groups had decrease in salivary cortisol, but only the treatment group had moderate evidence ($p=0.06$) of a decrease beyond chance. No significant result was obtained when comparing the difference between the placebo and the treatment group.

4. Discussion and Conclusion

After a week of bi-daily dosing the effect of alpha-methyldopa on sympathetic activity was evaluated. Significant results were observed in a number of parameters. Comparing the placebo group with the treatment group statistical significance was observed in CSI, RRSD, QT interval and in all the pulse rates measured with the different apparatus. RRSD did have a significant result however the decrease in value is an indicator of sympathetic predominance which is contradictory to the mechanism of action of methyldopa. A limitation to the study was the small sample size. Parameters that did not have significant results included, BP, QRS complex, QTc, skin conductance and salivary cortisol. Since methyldopa is an anti-hypertensive agent, a decrease in blood pressure was observed but it was not significant, possibly because the subjects that participated in the trial were young, healthy individuals. Skin conductance and salivary cortisol concentration did not decrease as expected but no significant increase was observed either.

Adverse events were more frequent in the treatment group compared to the placebo group. Dizziness and drowsiness were both observed in 20% of the group while lack of concentration and orthostatic hypotension were each observed in 10% of the group. Alpha-methyldopa is an alpha-adrenergic agonist that inhibits sympathetic outflow. Alpha-methyldopa decreased BP, Pulse, CSI, RRSD and QTc whereas it increased QRS complex, QT interval, skin conductance and salivary cortisol. Most of the parameters indicated suppression in sympathetic activity with the exception of RRSD, skin conductance and salivary cortisol concentration. Some limitations to the study include the small sample size and the parallel study design. A crossover study design may have been more informative. It remains to be seen if methyldopa has any significant role in the treatment of ADHD as this will warrant further studies. Its effect on the sympathetic system was highlighted in this study.

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