HOT FLUSHES, VASCULAR REACTIVITY AND THE ROLE OF THE ALPHA-ADRENERGIC SYSTEM

J Sassarini, MBChB[1], H Fox, MBChB[1], W Ferrell, MBChB PhD FRCP[2], N Sattar, FRCP, FRCPath[3], MA Lumsden, BSc, MBBS, MD[1].

[1]Centre for Population & Health Sciences, University of Glasgow, United Kingdom, G11 6NT; [2]Institute of Infection, Immunity and Inflammation, University of Glasgow, United Kingdom, G11 6NT; [3]Institute of Cardiovascular and Medical Sciences, University of Glasgow, United Kingdom, G12 8TA

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Corresponding Author: Dr. Jenifer Sassarini

jenifer.assarini@glasgow.ac.uk

Level 2, McGregor Building, Western Infirmary, Dumbarton Road, Glasgow, G11 6NT

Tel: (141) 211 2327, Fax: (141) 553 1367

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Abstract

Background: 70% of postmenopausal women suffer from hot flushes but the pathophysiology is poorly understood. Proposed mechanisms include altered peripheral vascular reactivity and a narrowed thermoneutral zone. A trigger has not yet been identified, however the alpha-adrenergic system and specifically noradrenaline has been implicated

Aim: Assess the role of the alpha-adrenergic system by studying the effect of clonidine (alpha-adrenergic agonist) on flushes and cutaneous microvascular perfusion.

Methods: 32 postmenopausal women with severe flushing and 14 non-flushing postmenopausal women were recruited. Cutaneous microvascular perfusion was measured using laser Doppler imaging and endothelial function was assessed by iontophoresis (administration of vasoactive agents through the skin by an electric current) of acetylcholine (ACh – endothelium dependent) and sodium nitroprusside (SNP – endothelium independent). In a double-blind, longitudinal crossover study, clonidine (an alpha-adrenergic agonist) was compared to placebo in its ability to modulate this response in the flushing group of women.

Results: The response of the subcutaneous vessels was greater in women who flushed than those who did not, (ACh; $p<0.001$ and SNP; $p=0.001$). However, even though the intensity and number of flushes was decreased by clonidine, there was no difference compared to placebo ($p=0.21$) and this 'placebo effect' was also noted in perfusion responses (ACh, $p=0.98$, SNP, $p=0.50$).

Conclusion: There was a significant 'placebo effect' for both clinical response and the reactivity of the sub-cutaneous vessels making conclusions regarding the role of the alpha-adrenergic nervous system in hot flushing difficult to determine at a peripheral level. The mechanism for the change in vascular reactivity remains unclear.

INTRODUCTION

Hot flushes are the most commonly reported symptom in postmenopausal women, occurring in approximately 73% of women (1) causing significant morbidity in 25%, affecting social life and even the ability to work (2). With improved healthcare and increased life expectancy (death rates decreased by 19% in the last 10 years), women spend a considerable proportion of their lives (30 years on average) after the menopause. At present 36% of the women in the UK are over 50 years of age. If left untreated, hot flushes resolve within one year, or less, in the majority of postmenopausal women. A third will report symptoms that last up to 5 years after natural menopause, and in 20% hot flushes persist for up to 15 years. This equates to as many as 1.5 million women in the UK.

Forty percent of these women will seek medical advice for the management of hot flushes and other related symptoms, and these have been successfully treated with oestrogen for years. In 1995, 37% of American women took HRT, principally for this purpose. For this reason few studies were carried out investigating the pathophysiology of flushes. However, following publication of results from studies such as the Women's Health Initiative and Million Women Study there has been renewed interest in this topic since HRT prescription dropped 50%.

Hot flushes are periods of intense heat which are associated with sweating and peripheral vasodilation, and whilst the mechanism is still poorly understood, hypotheses exist surrounding both central and peripheral mechanisms.

Studies by Freedman et al, using an ultrasensitive temperature probe, suggest that hot flushes are triggered by small elevations in core body temperature (Tc) acting within a narrowed thermoneutral zone in symptomatic postmenopausal women (3). This group found that small but significant elevations in Tc precede most (76%) hot flush episodes (3-5). These same investigators subsequently found that postmenopausal women with hot flushes had a narrower thermoregulatory zone (0°C) compared with postmenopausal women who do not flush (0.4°C). This narrowing was mainly due to a

lowering of the sweating threshold in symptomatic women (6). Since heat loss mechanisms can be triggered by a 0.01^{o} elevation of core body temperature above the regulatory zone, the subtle changes in temperature before a hot flush, coupled with a narrow homeostatic temperature zone, may trigger the heat loss mechanisms that lead to hot flush symptoms.

Norepinephrine is thought to be the primary neurotransmitter responsible for lowering the thermoregulatory set point and triggering hot flushes (6-7). Animal studies have shown that intrahypothalamic injection of norepinephrine acts to narrow the thermoregulatory zone (8). It has also been shown that plasma levels of the norepinephrine metabolite 3-methoxy-4-hydroxyphenylglycol (MHPG) are significantly increased both before and during hot flush episodes in postmenopausal women (4). Hot flushes can be provoked in symptomatic postmenopausal women with the α_2-adrenergic antagonist yohimbine, and ameliorated with clonidine, an α-adrenergic agonist (9). It has also been demonstrated that clonidine acts to widen the thermoregulatory zone in humans (10-11).

Clonidine is an α_2-adrenergic agonist which is licensed for the treatment of hypertension, migraines and postmenopausal vasomotor symptoms. Clonidine is thought to exert its hypotensive effect through stimulation of α-adrenergic receptors in the vasomotor centre of the medulla (12). It has also been shown to have a beneficial effect on menopausal flushing (13-15), in particular by decreasing the intensity of the flushes. The mechanism is not fully understood, however it may exert its effect on hot flushing through a reduction in peripheral vascular reactivity (16) and also through central mechanisms.

Peripheral vascular reactivity, as assessed by laser Doppler imaging and iontophoresis (discussed in detail in materials and methods), has been shown to be significantly increased in postmenopausal women who flush when compared to matched women who do not (17). We, therefore, aimed to study the role of the alpha-adrenergic system at a peripheral level utilising clonidine and its effect on flushing.

MATERIALS AND METHODS

Study Participants

A total of 32 postmenopausal women who each experience at least 20 flushes/day and 14 non-flushing women were recruited to participate in this study. Recruitment of volunteers followed Scottish media coverage, as well as from gynaecology and menopause clinics. A number of women were also recruited from the West of Scotland Breast Screening Centre in Glasgow.

Participating women are all aged 50-65 years, non-smokers, not known to be hypertensive, non-diabetic and not taking any drugs which could affect vascular function. Menopausal status was determined by either an FSH greater than 20 Units/Litre or amenorrhoea for 1 year or longer.

Design and Procedures

This was a double-blind, cross-sectional, longitudinal study of crossover design. Study participants were all seen at baseline when skin blood flow was assessed using laser Doppler imaging (LDI) with iontophoresis (ION) of vasoactive compounds. Participants were randomised in a double-blind manner to receive either Clonidine 0.1mg/day or placebo. Baseline microvascular measurements were taken, and after 4 weeks of treatment, microvascular measurements were assessed as before. The participants were then crossed over to receive the alternate treatment for 4 weeks and microvascular measurements performed for the final time. A washout period between treatments was not required as Clonidine has a short half life and any effects upon peripheral vascular reactivity are likely to be rapid. Qualitative measures were obtained at the same time points.

Each participant was asked to keep a ‘Hot Flush’ diary for 3 weeks prior to initial assessment and throughout the study. This documented number of flushes, severity of flushes on a scale of 1 to 3 and number of times that participants were awake through the night secondary to flushing. This was

recorded on a daily basis and at the end of each 7 day period, participants were also asked to complete a hot flush related daily interference scale (HFRDIS), which subjectively assessed their perception of the affect flushing had on a number of daily activities.

Assessment of cutaneous microvascular perfusion in the control group was made at baseline and at 4 weeks. This group did not receive any treatment.

All work was performed according to the Declaration of Helsinki with approval granted by the institutional ethics committee (REC 01/50704/43). All patients gave written informed consent.

MEASUREMENT OF CUTANEOUS MICROVASCULAR PERFUSION

Cutaneous microvascular responsiveness can be assessed non-invasively by combining laser Doppler imaging (LDI) with iontophoresis (ION) (18).

Iontophoresis is a technique which allows for transdermal delivery of vasodilator agents acetylcholine (ACh) and sodium nitroprusside (SNP) across the skin under the influence of an applied current. In the past iontophoresis has been used in conjunction with laser Doppler flowmetry, a non-invasive method for assessing microvascular perfusion at a single point (19). More recently, iontophoresis has been combined with LDI, which reduces measurement variability (20-21). This is because unlike laser Doppler flowmetry, laser Doppler imaging measures perfusion across many points (22), and an average measure of perfusion can be computed for any chosen area.

Iontophoresis of acetylcholine (ACh) at the anode tests endothelial function since its vasodilator action involves binding to muscarinic receptors on endothelial cells, with subsequent generation of NO. It is therefore said to be ‘endothelium dependent’. Vasodilatation is ultimately mediated by action of NO on vascular smooth muscle (via the cGMP pathway) and so iontophoresis of an NO donor, sodium nitroprusside (SNP), delivered at the cathode, is used as an ‘endothelium-independent’ control to test the integrity of vascular smooth muscle.

Drug delivery is achieved using a battery-powered constant-current iontophoresis controller (MIC-1e; Moor Instruments Ltd., Axminster, U.K.). The chambers used for iontophoresis (ION 6; Moor Instruments Ltd.) are constructed of Perspex (internal diameter 22mm; area 3.8cm^2) with an internal platinum wire electrode. Two chambers are attached to the skin of the volar aspect of the forearm by means of double-sided adhesive discs, avoiding hair, broken skin, and superficial veins. The chambers are connected to the anode and cathode connections on the iontophoresis controller and the voltage across the chambers is monitored. A thermometer is also attached to the arm in order to measure skin temperature.

2.5ml of 1% ACh (Sigma) is introduced to the anodal chamber and 2.5ml of 1% SNP (Sigma) is introduced to the cathodal chamber. The vehicle for these drugs is 0.5% sodium chloride (NaCl) in order to reduce electrically-induced artefacts (23). Both of these agents are delivered simultaneously during each period of current administration. Fluid is prevented from escaping by placing circular 32mm coverslips over the chambers.

The iontophoresis protocol involves incremental current delivery with four scans at 5µA, four at 10µA, four at 15µA and two at 20µA, giving a total charge of 8mC.

The laser Doppler imager (Moor Instruments, UK) is equipped with a red laser (wavelength 633nm, power 1mW, beam diameter 1mm). The laser is scanned in a raster fashion over both chambers and through the coverslips. The backscattered light is collected by photodetectors and converted into a signal proportional to perfusion in arbitrary perfusion (flux) units (PU) that is displayed as a colour-coded image on a monitor. Perfusion measurements are obtained using the imager manufacturer's image analysis software by outlining a region of interest (ROI) around the internal circumference of the chamber. Statistical analysis of the ROI is subsequently performed to yield the median flux value across approximately 700 measurement points. Twenty repetitive scans are taken during each LDI + ION assessment, the first being a control (before current administration), followed by the incremental current protocol as described above (fourteen scans), and followed by a further five scans with no

current administration. An assessment of the overall response to the drugs is obtained by calculating the area under the curve (AUC).

This technique is reproducible with between-day and within-day coefficients of variation of 6.4 ±3.3% and 8.9 ±5.3% respectively, variability being reduced by averaging perfusion over a large skin area (23).

All participants fasted for at least 5 hours prior to assessment (water only permitted). Prior to the procedure, patients were allowed to acclimatize for 15 minutes in a temperature-controlled room. Participants all lie in a semi-recumbent position with the flexor aspect of the forearm exposed on an arm rest.

STATISTICAL ANALYSIS

Measurement of vascular responses was performed using raw values. Comparisons were by General Linear Model. Because of the marked differences in variances between basal perfusion values and the maximal responses to the drugs, but the similar coefficients of variation, $\log_{10}$ transformation of the data was performed to equalise the variances and thereby permit parametric data analysis

For comparison of clonidine and placebo treatment, neither log nor square root transformation resulted in a Gaussian distribution. Therefore graphs are presented as raw – baseline perfusion values and analysis of the data was by Mann Whitney analysis of the corrected area under the curve (AUC); that is the AUC calculated from raw values with the baseline data subtracted.

For analysis of basal perfusion values for flushers vs controls, again the data were non-normally distributed, therefore analysis was carried out using the Kruskal-Wallis test.

Comparison of demographic data was by T-test as data was of a Gaussian distribution.

Diary data and HFRDIS were compared using Mann Whitney analysis of total number of flushes per day, hot flush score, night time awakening and HFRDIS.

RESULTS

32 women with severe hot flushing, aged between 50 and 65, who were medically fit and were not taking drugs that might impact on vascular reactivity, were recruited and had LDI + ION assessment of vascular reactivity of the subcutaneous vessels.

Full demographic criteria were available for 29 women with flushing. (Table 1).

Following initial assessment, 7 women declined continued participation in the study; therefore there are data for 25 women comparing clonidine to placebo.

Responses to acetylcholine (ACh) and sodium nitroprusside (SNP)

Vascular reactivity for 32 flushers was measured using LDI. Data were analysed as described above.

The response of the cutaneous vessels was greater in women who flushed than in those who did not. The enhanced vascular response occurred following administration of both the endothelium-dependent (ACh) and independent vasodilators (SNP), (ACh, $p = < 0.001$, SNP, $p = 0.001$, 2-way ANOVA) (17).

There was no difference in the response of cutaneous vessels when comparing clonidine to treatment with placebo for either the endothelium dependent or independent responses ($p=0.98$ and $p=0.50$ respectively, Mann Whitney) (Figure 1).

However, there was an increase in endothelial dependent perfusion responses seen for both placebo and clonidine when compared to baseline. Clonidine vs baseline, ACh, $p=0.04$ and SNP, $p=0.29$. Placebo vs baseline, ACh, $p=0.01$ and SNP, $p=0.06$ (Figure 1).

Whilst it would be impossible to determine that the drug delivery to each is identical, we can address the question of differences in vascularity in terms of basal perfusion values. If there is no difference in the absolute perfusion values between the groups prior to iontophoresis, then it is likely that there is no substantial difference in vascularity – either in terms of the number of vessels or rate of flow.

Analysis of groups indicates no difference in pairwise comparisons i.e. ACh control vs ACh flush and SNP control vs SNP flush.

Subjective measurements

Although the number and severity of flushes decreased with clonidine, there was no statistically significant improvement in either the number or severity of flushes when comparing clonidine to placebo (P=0.21). There was no improvement in night time awakening or hot flush related daily interference (see Table 2).

DISCUSSION

In this group of postmenopausal women, those with severe flushing have a greater vasodilator response than those women with no flushing (17). This is in keeping with previous studies showing a diminished vasoconstrictor response, and increased blood flow during a hot flush (24-25).

Dilation of cutaneous vessels, with resultant increase to blood flow, is a hallmark of flushing. This resembles a heat dissipation response, whereby increases in core temperature trigger nonevaporative heat loss through vasodilation of subcutaneous vessels. In the rat, cutaneous vasodilation of the tail is a primary mechanism of thermoregulation and it has been shown that after ovariectomy, tail skin temperature increases, and that this effect can be abolished by treatment with oestrogens (26). Furthermore, castration in rats is associated with increased hypothalamic norepinephrine levels (27) and oestrogen replacement decreases turnover (28).

Monoamines, especially norepinephrine (NE), have been shown to play an important role in the control of thermoregulation. Noradrenergic stimulation of the pre-optic area of the hypothalamus in monkeys (29) and baboons (30) by microiontophoretic application of NE causes peripheral vasodilation, heat loss and a drop in core temperature, similar to changes which occur in women during hot flushes.

This is consistent with NE involvement in hot flushes, which is further supported by clinical data showing that clonidine, an ά2-adrenergic agonist that reduces brain NE, reduces hot flush frequency. Yohimbine, an ά2-adrenergic antagonist has also been shown to provoke hot flushes(9).

Whilst clonidine did decrease the number and intensity of flushes in our group of women, this was not significant when compared to placebo. This is perhaps not surprising as a recent meta-analysis examining ten trials comparing clonidine to placebo found only 4 showed an improvement in number and severity of flushing and 2 out of 4 were of poor quality (31).

What is surprising, perhaps, is the increase in vascular reactivity seen with both clonidine and placebo when compared to baseline measurements, with no difference between the two treatments.

The placebo effect is well documented, and in randomised controlled trials (RCTs) is designed to assess the efficacy of the potential treatment. The magnitude of the placebo response is found to be partly dependent on the condition. In studies of anti-depressant medications, placebo response rates average approximately 30%, ranging from 12% to more than 50% and in RCTs of hormone therapy for vasomotor symptoms in menopause, the placebo response rate averages 51% (32). This may explain the subjective diary results and it has been suggested that if harnessed reliably, the placebo response, may enhance treatment outcomes (33).

The increase in cutaneous microvascular perfusion seen after 4 weeks of treatment with placebo is more difficult to explain. The placebo effect is well recognised and long studied in analgesia and it was in 1978 that endogenous opioids were first shown to be involved in placebo analgesia. Levine et al demonstrated that the opiate antagonist naloxone was able to reduce the placebo response in dental postoperative pain (34). Since then, placebo and placebo-related effects have been analyzed and specific mechanisms at both the biochemical and cellular level have been uncovered. Benedetti has conducted an extensive review of a number of these mechanisms, and amongst them is a reduction of β-adrenergic activity of the heart, changes of metabolic responses in different brain regions (possibly inhibition of serotonin reuptake), as well as modulation of immune factors including IL-2 and IFN-γ (35). It is therefore possible that placebo treatment could affect peripheral vascular reactivity, but this would clearly require further study.

Equally, the peripheral effects of clonidine are not clear. Ginsburg et al, found that an increase in forearm blood flow induced by intravenous infusion of adrenaline, angiotensin and noradrenaline was significantly less in women treated for at least 6 weeks with clonidine compared with that induced in the women by infusions given before treatment. (36). However, our results would seem to contradict these. One possible explanation is that the treatment duration in our study (4 weeks) was insufficient

to elicit peripheral vasoconstriction. However, a more likely explanation may relate to differences in methodology. Ginsburg used venous occlusion plethysmography to measure hand and forearm perfusion but, for the forearm, overall change in perfusion will be much more strongly influenced by change in blood flow to the forearm muscles, rather than the skin. However, the LDI + ION methodology only assesses skin perfusion, and is therefore more appropriate for studies of flushing. Ginsburg et al found that constrictor responses in the hand were unchanged. Hand perfusion would be less affected by muscle blood flow as there are fewer muscles, and may therefore be more comparable with our results.

One could argue that a 4 week duration following commencement of clonidine was too long to see the peripheral vasoconstriction that might be elicited by the treatment as this anti-hypertensive agent has a dual action. When first administered, clonidine stimulates peripheral ά1-adrenoceptors (ARs) resulting in vasoconstriction, but subsequently acts on the central ARs to inhibit sympathetic drive resulting in vasodilation (37). The central action predominates over the peripheral, therefore perhaps any initial decrease in cutaneous microvascular perfusion that there may have been is now overridden by the centrally mediated and predominant vasodilatory effect.

This may in fact, explain the increase in perfusion responses seen in our group of women after 4 weeks of clonidine (Figure 2), and may explain why anecdotally there often appears to be an initial improvement in symptoms followed by a return of vasomotor symptoms.

However, clonidine is used for postoperative shivering because it is thought that, like general anaesthetic agents and sedatives, it decreases shivering thresholds by a generalised impairment of central thermoregulatory control (10). These same authors also demonstrated that clonidine administration increased the sweating threshold. They went further to suggest, that as the thermoregulatory effects of clonidine resembled those of volatile anaesthetics, it was likely that the alteration in the shivering and sweating thresholds were as a result of central thermoregulatory inhibition (11).

CONCLUSION

There was a significant 'placebo effect' for both clinical response and the reactivity of the subcutaneous vessels making conclusions regarding the role of the adrenergic nervous system in hot flushing difficult to determine at a peripheral level. The mechanism for the change in vascular reactivity is unclear.

Limitations

These were all lean, Caucasian women; therefore care must be taken when extrapolating these results to the general population.

ACKNOWLEDGEMENTS

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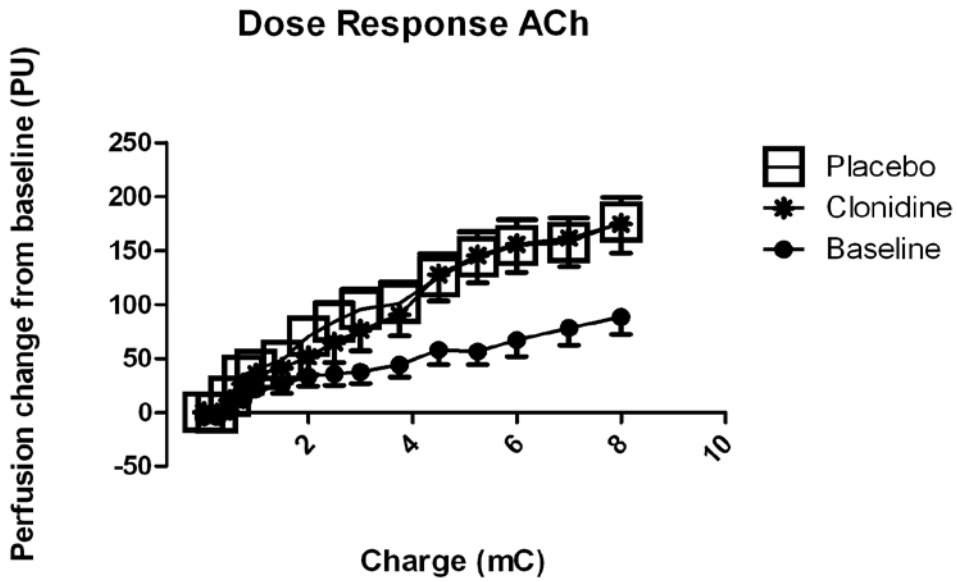
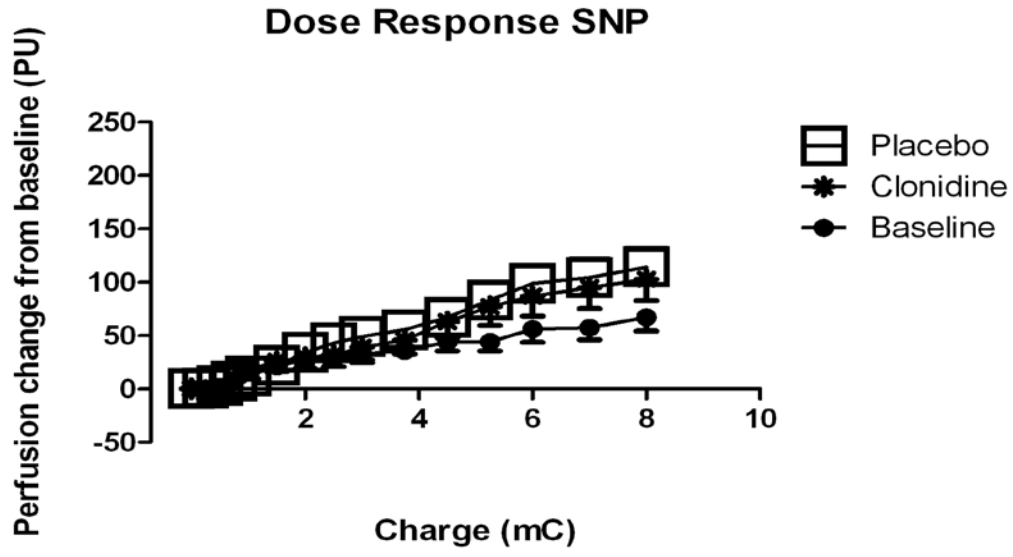
CONFLICTS OF INTERESTS

The authors report no conflict of interest.

Figures



A.



B.

Figure 1: Dose Response Curves
Flux raw – baseline values (perfusion units) with increasing charge for acetylcholine (A) and sodium nitroprusside (B) in flushing women when treated with clonidine and compared to treatment with placebo. Data are mean ± SEM. As described in text, data are analysed as AUC and compared by Mann Whitney. P values for clonidine vs placebo are 0.98 and 0.50 for Ach and SNP respectively. Clonidine vs baseline, ACh, P=0.04 and SNP, P=0.29. Placebo vs baseline, ACh, P=0.01 and SNP, P=0.06.

Tables

	Participants
n	29
Age	56 (53-61)
Years since LMP	8 (1-12)
BMI	25.1 (24.3-28.1)
Parity	2 (0-3)
Smoking	0
*SBP	126.1 ± 2.67
*DBP	70.48 ± 2.11

Table 1: Demographic characteristics of study subjects
Data are median (interquartile range). All women are non-smokers and normotensive.

				P values (Mann Whitney)		
	Placebo	**Clonidine**	**Baseline**	**P vs C**	**C vs B**	**P vs B**
hot flush/day	5.16 (2.64,18.75)	4.43 (0.68, 19.39)	6.05 (3.14, 17.62)	0.21	0.02	0.25
NTA	2.68 (0.43, 5.32)	2.46 (0.58, 4.31)	2.50 (0.63, 4.71)	0.45	0.79	0.73
HFRDIS	37.25 (2.67, 89.25)	33.75 (5.00, 100.00)	44.17 (8.50, 89.00)	0.5	0.43	0.7
Hot flush score	11.93 (4.43, 56.25)	8.87 (0.62, 58.18)	12.14 (5.70, 52.86)	0.13	0.01	0.4

Table 2: Statistical comparison of placebo, clonidine and baseline

Flush/day = total number of flushes/week divided by the number of diary days completed, and a mean taken of the weeks completed. Hot flush score = mean daily flush frequency multiplied by mean score. A mean was taken of the weeks completed. NTA (night time awakening) = total number of NTA/week divided by the number of diary days completed, and a mean taken of the weeks completed. HFRDIS (hot flush related daily interference scale) was completed once per diary week, and a mean was taken of the weeks completed. Placebo and Clonidine weeks, diaries were completed for 4 weeks each, and baseline diaries were completed for 3 weeks.

Data are P values determined by Mann Whitney analysis of mean values as demonstrated in table 2.

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