Original Article

Amlodipine alters hemorheological parameters: Increased efficacy at the cost of edema?

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ABSTRACT

Background: Despite several decades of use of calcium channel blockers, the side effect of edema persists as a class effect, and its mechanism is unresolved. Amlodipine has effects on hemorheology (HR), and its hemodilutory property may partly contribute to its antihypertensive action. This aspect is not well studied, and the literature is sparse in this regard.
Objective: This experiment was planned to determine effect of a single-dose administration of amlodipine on HR parameters in normal human volunteers.
Methods and results: Amlodipine (5 mg) or S (-) amlodipine (2.5 mg) was administered to 27 normal human volunteers. Whole-blood viscosity (WBV) at different shear rates, plasma viscosity (PV), red cell rigidity (RCR), red cell aggregation (RCA), hematocrit (Hct), plasma hemoglobin, along with plasma drug concentration were determined at time intervals, $t = 0$, 4, 8, 12, and 24 h. Statistically significant reductions were observed at $t_{max} = 4$ h in WBV at shear rates of 0.512 s^{-1} ($p < 0.005$), WBV at shear rates of 5.26 s^{-1} ($p < 0.01$), PV ($p < 0.05$), and Hct ($p < 0.01$). At $t = 8$ h, as drug concentration reduced, some of the changes persisted and later slowly decreased with the decreasing drug concentration till $t = 24$ h. Red blood cell—related parameters such as RCA and RCR remained unaltered. WBV values at all shear rates, when corrected for Hct = 0.45, did not show deviation from their original values at any time.
Conclusions: Amlodipine causes a reduction in Hct and blood viscosity, along with hemodilution. These effects persist as long as the drug remains in plasma. Edema resulting from chronic dosing may be explained by the aforementioned effects. It is possible that antihypertensive action of the drug may be due to a combination of vasodilatation and an improvement in the HR properties.

1. Introduction

Clinical hemorheology (HR) studies in the past three decades have proved that hypertension (HT) is accompanied or preceded by an abnormal enhancement in one or more of the HR parameters such as whole-blood viscosity at low rate (WBV$_l$) or whole-blood viscosity at high shear rate (WBV$_h$), plasma viscosity (PV), red cell rigidity (RCR), red cell aggregation (RCA), plasma fibrinogen (PFIB), and hematocrit (Hct).[1–3] The nature of association between HT and HR is still debated on, with analogous descriptions stating that the association is like that of a "chick and embryo" or like "two chicks from the same embryo", or "neither chick nor embryo".[4,5] Nevertheless, concurrent occurrence of HT and elevated HR parameters is a well-known phenomenon. It is also expected that any therapy for HT would improve the HR profile, along with the lowering of elevated BP.[6] However, these findings are yet to influence the clinical research and management of HT, as outlined in the Joint National Commission (JNC)[7] and the World Health Organization/International conference on harmonisation of technical requirements for registration of pharmaceuticals for human use (ICH) guidelines on HT.[8]

Amlodipine, owing to its unique pharmacological, pharmacokinetic (PK), and pharmacodynamic profile of high oral bioavailability (BA), half-life between 30 and 50 h enabling once-a-day regimen, minimum peak-to-trough fluctuations, and low cost, is the most common calcium channel blocker (CCB) used in the

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treatment of essential HT.[9] While the effects of CCBs, in general, and amlodipine, in particular, on cardiac muscles and vasculature have been extensively studied, their effects on blood rheology have not received sufficient attention. HR effects of amlodipine have been reported in only one study to date,[10] with no follow-up studies to further evaluate its effects. In this study by Linde et al, amlodipine was found to increase RBC deformability.[10] However, the effects of amlodipine on various HR parameters in normotensives and at various concentrations and its effect on WBV at different shear rates have not been studied. The partitioning of the drug in plasma and erythrocytes and the mechanism underlying reduction in HR parameters are other important aspects, which need to be explored. We, therefore, conducted this study with the objective of evaluating these questions through an interdisciplinary approach involving clinical HR, pharmacological and PK parameters.

2. Methods

The study was designed as an analyst-blind, open-label, balanced, single-dose, PK study of amlodipine under fasting conditions in healthy human volunteers. While the PK study and measurement of plasma drug concentrations were conducted at Drug Monitoring Research Institute (DMRI), Mumbai, the determination of hematological and HR parameters was carried out in the Clinical Hemorheology Laboratory of School of Biosciences and Bioengineering, Indian Institute of Technology (IIT), Bombay. Young male volunteers (age 20.1 ± 2.2 years), free from any cardiovascular, cerebral, or renal diseases were selected. Smoking and consumption of alcohol and medicines other than the drug during the study period was strictly forbidden. The volunteers were made to adhere to the plan of diet, rest, and consumption of medicine under study.

A total of 27 normal volunteers were included in this in vivo study. This was a stand-alone study and not an extension of a BA/bioequivalence (BE) study. The final sample size in our study was determined by the number of volunteers who were available for screening and inclusion within the timeframe stipulated for initiating the study. While no power analysis was performed during the initiation of the study, the number of subjects in our study matches the usual sample size for BA/BE studies which evaluate drug concentration levels with a sample size power at 80% or higher.[11] After an overnight fasting period of at least 10 h, they were administered a single dose of one tablet of amlodipine (5 mg) along with 240-ml drinking water. The plasma drug estimations were carried out by withdrawal of blood samples at $t = 0, 2, 3, 4, 5, 6, 7, 8, 10, 12, 16, 24, 48, 72,$ and 96 h. HR parameters such as WBV at 18 shear rates ranging from 0.512 to 94.5 sec^{-1}, PV, RCR, RCA, and Hct were determined at $t = 0, 4, 8, 12,$ and 24 h. A physician carried out a clinical examination of the subjects at the time of check-in and checkout, while vital signs and adverse effect monitoring was performed throughout the study, from the prestudy day to 96 h after administration of dose.

2.1. PK and statistical evaluation

Amlodipine plasma levels were processed using HMS software (E Merck, USA). PK parameters such as AUC $_{0-t}$, AUC $_{0-inf}$, Kel, $t_{1/2}$, C_{max}, and t_{max} were calculated for each volunteer. Amlodipine plasma level data and PK parameters were statistically analyzed using SPSS software. MS Excel was used for calculation of averages, standard deviation, and student's t-test.

2.2. Independent ethics committee approval

The independent ethics committee attached to DMRI, Mumbai, approved the protocol and all the amendments. The inclusion of volunteers in the study was subject to their consent, which was documented on the informed consent form for the study.

2.3. Determination of HR and hematological parameters

All the HR measurements were performed at the Clinical Hemorheology Laboratory of School of Biosciences and Bioengineering, IIT, Bombay, in accordance with the norms specified by the International Committee for Standardization in Hemorheology.[12] The procedure and the instruments described below for determination of various HR parameters have been used in our laboratory for the past 15 years and have been reported in several previous publications.[13—15]

About 10—12 ml of blood was withdrawn from the antecubital vein using a plastic disposable syringe with a 21-gauze stainless steel needle applying minimum suction. The blood was immediately transferred to a plastic vial containing a solution of sodium salt of ethylenediaminetetraacetic acid (15 mcg/ml of blood). The determination of HR and hematological parameters was completed within 4 h of withdrawal of the blood sample.

A Low-Shear 30 viscometer (Contraves, Zurich), specially designed for the rheological measurements of small volumes of biological samples, was used for determination of WBV and PV. Blood (mixed with anticoagulant) was sheared in the gap between a cylindrical bob and a coaxial rotating cup. The resistance to the rotating cup is proportional to the shear stress in the fluid; the amount of torque produced by resistance was indicated on the digital display of the instrument. This reading was converted into viscosity in centipoises by multiplication with a factor. Each blood sample was subjected to 18 different shear rates from 0.512 s^{-1} to 94.5 s^{-1}. Whole-blood viscosity determined at 51.2 rpm was designated as WBV_h, while that determined at 0.512 s^{-1} was designated as WBV_l and at 5.26 s^{-1} was designated as WBV_m. After the determination of WBV, the blood sample was centrifuged at 3000 rpm for 15 min and maximum possible volume of plasma was separated from it, taking care not to disturb the cellular layer. PV was determined on the Low-Shear 30 viscometer in a similar manner at shear rates of 20.4, 51.2, and 94.5 s^{-1}.

RCA was determined indirectly by using the following formula[16]:

$$RCA = \frac{(WBV_l)^{45/Hct}}{(PV_{20.4})}$$

where WBV_l is the whole-blood viscosity at 0.512 rpm, $PV_{20.4}$ is the plasma viscosity at 20.4 rpm, and Hct is the hematocrit.

Immediately after the separation of plasma from the centrifuged sample of blood, a suspension of red cells was prepared by pipetting out 500 μl from the middle pack of the red cells and suspending them in 1 ml of Ringer buffer solution. The viscosity of red cell suspension was determined at 94.5 rpm. Its Hct was determined with the help of an autoanalyzer (Sysmex, Japan) by the RBC pulse height detection method.[17,18] The viscosity of the Ringer buffer solution was determined at the same condition and at the same rate of shear. RCR was calculated from the following formula[17]:

$$RCR = \frac{(\text{Viscosity of red cell suspension in ringer buffer solution})^{40/RHct}}{\text{Viscosity of ringer solution at the same shear rate}}$$

where RHct is the hematocrit of the red cell suspension.

The aforementioned equations for RCA and RCR have been used in several clinical studies pertaining to HR and hence have been used here.[16,17]

The magnitude of WBV has been shown by many workers to be a function mainly of Hct. Hence, a corrected value of WBV was calculated using the following formula[19]

$$WBV\_C = (WBV)^{45/Hct}$$

where WBV_C is the corrected whole-blood viscosity at shear rates, WBV is the apparent whole-blood viscosity at shear rates, and Hct is the hematocrit.

2.4. Determination of plasma amlodipine concentration

The blood samples, after phlebotomy, were immediately centrifuged at 10 °C and at 2500–3000 rpm for 10 min and stored at −20° ± 2 °C pending assay. Plasma amlodipine was estimated using a high-pressure liquid chromatography/mass spectroscopy method, developed and used by DMRI for their routine analytical studies. The method was validated in accordance with the principles of good laboratory practices. Sample preparation and analysis for the same has been performed as per Draft SOP/ANA/03/01 of DMRI. The criteria used for validation included specificity and selectivity, sensitivity, accuracy (relative recovery), precision (repeatability and reproducibility), percent extraction yield, and stability including freeze−thaw cycles, long-term stability, and bench-top stability. The data were processed by ANALYTE integrating system software.

3. Results

3.1. Effect of amlodipine administration on HR properties

The results of administration of a single dose of amlodipine on the HR parameters across time points in the subjects in the study are summarized in Table 1. Fig. 1 represents the changes in WBV_h, PV, RCR, WBV_m, WBV_l, RCA, Hct, and plasma drug concentration against time as brought about by amlodipine in systemic circulation. The mean drug concentration increased to a maximum (C_{max}) of 5.938 ng/ml at $t = 4$ h and then steadily declined to about 1.4 ng/ml at $t = 24$ h (Table 1 and Fig. 1). The changes induced by the drug did not totally disappear at $t = 24$ h as the drug was still in circulation.

As the concentration of amlodipine rose to its maximum at $t = 4$, WBV at high, low, or medium shear rates declined; the decline continued even up to $t = 8$ h even as drug concentration started rising. Except for WBV_l at $t = 4$, all changes in WBV values at $t = 4$ or 8 were statistically significant. Later, as drug concentration tended toward zero, WBV values too tended to rise and at $t = 24$ h, showed values lower (WBV_h) or somewhat larger (WBV_l, WBV_m) than their original values at $t = 0$. Changes in Hct and PV were found to be similar to those in WBV_h, although of a less magnitude. Variations in RCR and RCA were statistically insignificant at all time points and did not follow any specific pattern.
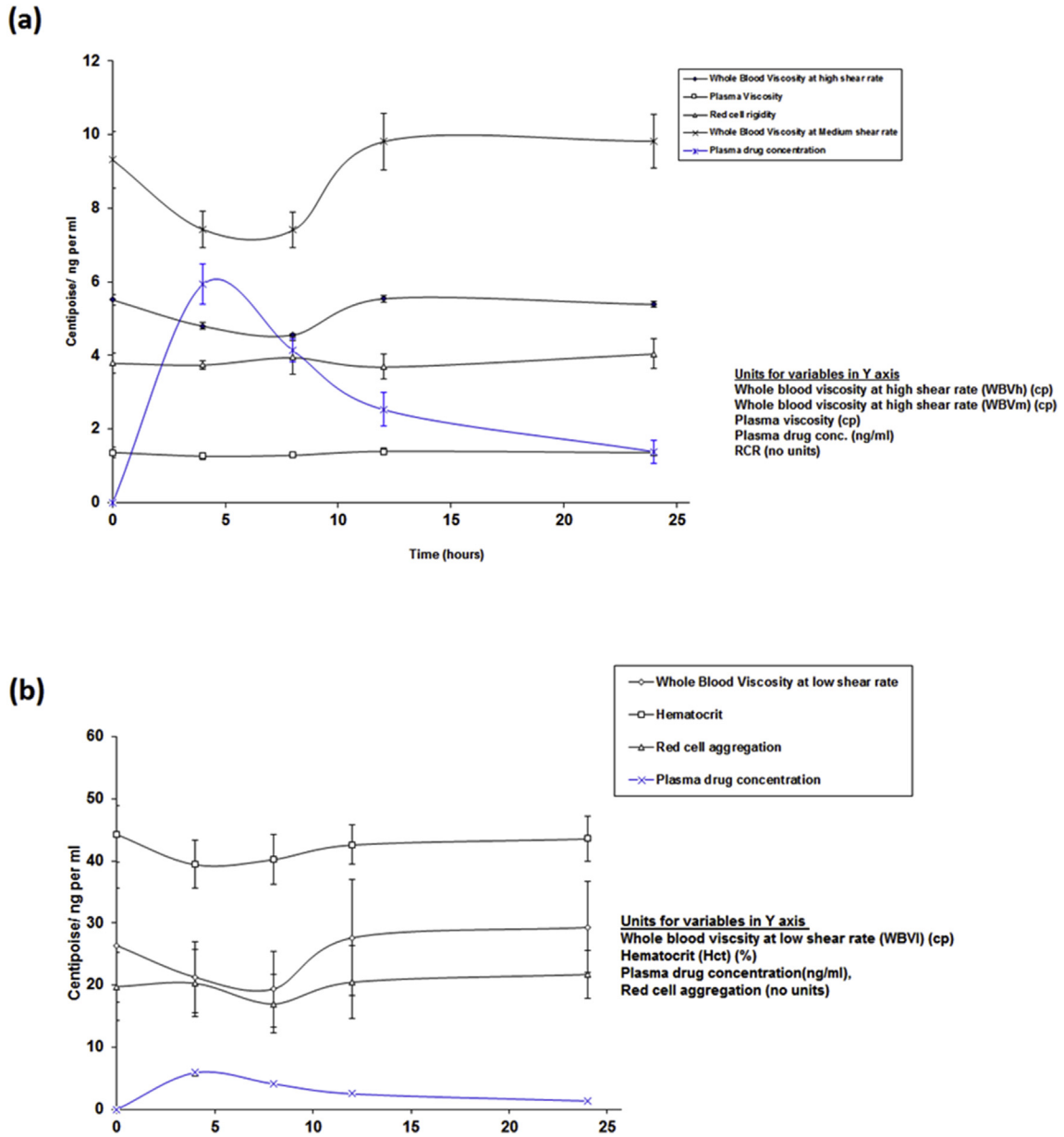
Fig. 2 shows the percentage reduction in the magnitude of various HR parameters at $t = 4$. The WBV values at low, high, or medium shear rates, when corrected for Hct = 0.45, showed much less reduction at $t = 4$ when compared with their uncorrected values. However, the WBV values corrected for Hct showed magnitudes between 4.16% and 9.24%, which could not be termed as negligible. Percentage changes at $t = 4$ for most HR parameters were found to be comparable to changes in Hct. However, the changes in RCA and RCR did not correspond well with the changes in Hct.

The average values obtained from regression analysis of plasma drug concentration and each of the HR parameters and the resulting Pearson's correlation coefficient values were also tabulated (Table 2). A strong negative correlation was found between the plasma drug concentration and uncorrected WBV at all shear rates and PV and for Hct and plasma drug concentration. Regression analysis for the plasma drug concentration with RCA and RCR showed poor correlation.

4. Discussion

All the values of HR parameters at $t = 0$ were in conformity with the normal values for the corresponding parameters as reported in previous studies.[20]

The most important findings were the statistically significant reductions in values of WBV_h, WBV_l, WBV_m, PV, and Hct after administration of drug at time $= 4$ h and/or $t = 8$ h. The drug concentration rises from zero at $t = 0$ to its maximum value (in HR studies) at $t = 4$ and falls slightly at $t = 8$ h. The causative factor for the reduction in these HR parameters is the reduction in Hct. It is interesting to note that the Hct reduces by 10.33% of its original value at $t = 4$ h. This reduction then tapers off to 8.18% at $t = 8$ h, 3.86% at $t = 12$ h, and 1.66% at $t = 24$ h. Thus, it reaches its minimum with the maximum drug concentration, and as the drug concentration reduces steadily, magnitude of Hct slowly comes back to its original value. However, even at $t = 24$ h, as long as the drug level

Table 1
Effect of amlodipine on HR parameters.

Time in hr/parameter	0	4	8	12	24
WBV_h (cp)	5.2 ± 0.78	4.80 ± 0.50***	4.56 ± 0.49***	5.44 ± 0.76	5.38 ± 0.72
WBV_l (cp)	26.42 ± 9.20	21.25 ± 5.72	19.34 ± 6.11*	27.65 ± 9.39	29.53 ± 7.37
WBV_m (cp)	9.32 ± 2.34	7.43 ± 1.44**	7.41 ± 1.46**	9.81 ± 2.02	9.83 ± 1.44
PV (cp)	1.36 ± 0.15	1.27 ± 0.88*	1.29 ± 0.39*	1.39 ± 0.096	1.37 ± 0.083
Hct (%)	44.35 ± 4.55	39.46 ± 3.84**	40.28 ± 4.03*	42.64 ± 3.24	43.62 ± 3.63
RCR	3.80 ± 0.28	3.75 ± 0.12	3.95 ± 0.45	3.69 ± 0.34	4.05 ± 0.40
RCA	19.78 ± 5.43	20.31 ± 5.46	16.99 ± 4.69	20.48 ± 5.94	21.73 ± 3.90
Plasma drug concentration (ng/ml)	0	5.94 ± 0.54	4.14 ± 0.32	2.54 ± 0.45	1.39 ± 0.32

HR, hemorheology; SD, standard deviation; WBV_h, whole-blood viscosity determined at 51.2 rpm; WBV_l, whole-blood viscosity determined at 0.512 rpm; WBV_m, whole-blood viscosity determined at 5.26 rpm; Cp, centipoises; PV, plasma viscosity; Hct, hematocrit; RCR, red cell rigidity; RCA, red cell aggregation.
Values were evaluated by two-tailed Student's 't' test with respect to $t = 0$.
Results were expressed as mean ± SD.
* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.005$.

Fig. 1. (a) Effect of time on various HR parameters and drug concentration. (b) Effect of time on various HR parameters and drug concentration. (Units: WBVh (cp), WBVm (cp), PV (cp), Plasma drug conc. (ng/ml), RCR (no units)). HR, hemorheology.

has not reached zero, reduction in Hct too is yet to reach zero. Except for WBV_l, the reduction in HR parameters is higher at $t = 4$ compared with $t = 8$ h.

The centrality of Hct reduction in improving HR parameters in normal controls is corroborated by the contrast in the changes occurring in WBV at various shear rates and their corresponding values corrected for Hct. While WBV_h, WBV_l, and WBV_m show statistically significant reductions at $t = 4$ h and $t = 8$ h, WBV_h_C, WBV_l_C, and WBV_m_C do not show changes of statistical significance. As the average Hct at $t = 0$ is 44.35, WBV_C values (which correspond to Hct = 45) are almost identical to their corresponding WBV values. However, the effect of reduction in Hct at higher t values is totally masked by conversion of WBV values to WBV_C values. Hence, WBV_C values remain relatively unaltered on passage of time. HR parameters related to RBCs, viz. RCR and RCA, do

not show much variation on time after administration of amlodipine. In this study, serum erythropoietin levels were not measured in the study subjects.

Because the Hct level at zero hour was measured and it was found to be within the normal range for the study population, the influence of erythropoietin in causing variations in Hct is unlikely. The reduction in Hct and the subsequent reduction in magnitude of selected HR parameters can be attributed to hemodilution. It is postulated that amlodipine, immediately after its administration, absorbs water from surrounding tissues into the blood stream, resulting in hemodilution. This hypothesis is supported by the almost parallel reduction in most HR parameters up to $t = 4$ or 8 h. After $t = 8$ h, all parameters tend to come back to their original values. The relatively low values of WBV_C compared with WBV also support this hypothesis. A strong or very strong negative
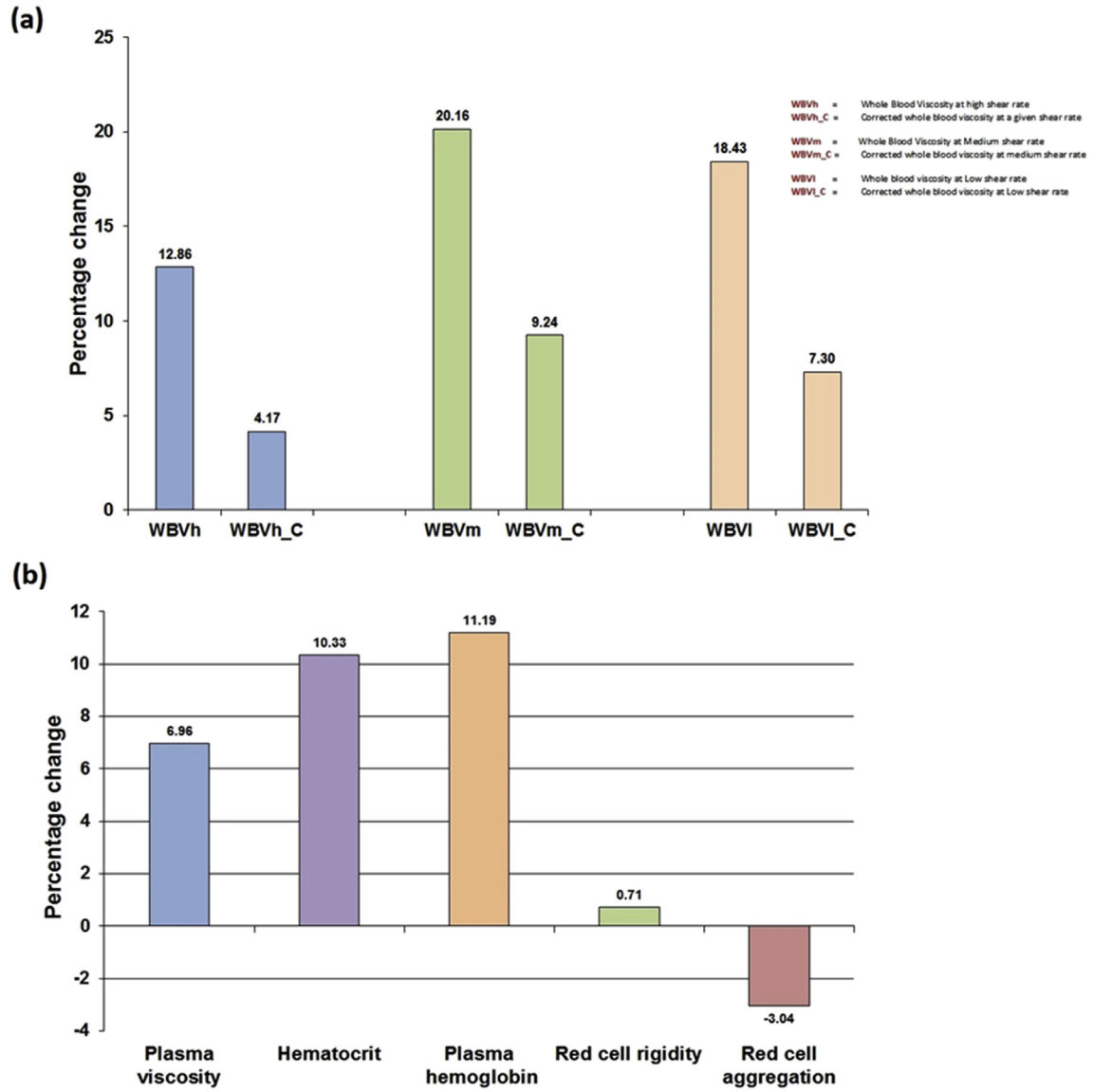
Fig. 2. (a) Percentage change in hemorheologic parameters at C_{max}. (b) Percentage change in hemorheologic parameters at C max (II).

Table 2
Correlation coefficient 'r' between the plasma drug concentration and HR parameters.

HR Parameter	Pearson's correlation coefficient (versus drug concentration)
Whole-blood viscosity at a high shear rate	−0.8
Whole-blood viscosity at a low shear rate	−0.76
Whole-blood viscosity at a medium shear rate	−0.81
Corrected whole-blood viscosity at a high shear rate	−0.41
Corrected whole-blood viscosity at a low shear rate	−0.58
Corrected whole-blood viscosity at medium shear rate	−0.69
Hematocrit	−0.98
Plasma viscosity	−0.83
Red cell aggregation	−0.21
Red cell rigidity	−0.31

HR, hemorheology.

correlation between drug concentration and plasma-related HR parameters (which can be diluted by hemodilution) further strengthens this hypothesis.

An earlier study found that after 4 months of amlodipine treatment, the total peripheral resistance index, WBV, Hct, and serum erythropoietin were found to decrease. The PV decreased, and the erythrocyte deformability increased in most patients, whereas no significant changes were observed in PFIB. The decrease in blood viscosity was attributed by the authors to hemodilution and a decrease in serum erythropoietin.[10] Later studies to substantiate

these findings do not exist. However, studies related to determination of HR effects of other CCBs reported an improvement in blood viscosity, i.e. decrease in various HR parameters. A study carried out in patients with HT using other CCBs for a prolonged period also supports the incidence of hemodilution caused by CCBs.[21] The administration of a single dose of amlodipine on selected hematological parameters has also shown to produce hemodilution.[22]

In an animal study conducted on spontaneously hypertensive rats, administration of intragastric amlodipine at a dose of 10 mg/kg for 6 weeks resulted in a significant decrease in mean blood pressure by 29% but had no effect on PV, PFIB concentration, RBC aggregation, and RBC deformability.[23] This study contradicts our finding, but this study was performed in rats, and dose of amlodipine used was very high (10 mg/kg), which may cause profound hypotension, leading to activation of counter regulatory mechanisms, such as the renin—angiotensin system.

Other antihypertensive drugs have also been shown to exert HR effects. In a study, beta-blocker, angiotensin converting enzyme (ACE) inhibitor, diuretic therapy, and calcium antagonist therapy have shown to alter WBV, PV, fibrinogen, and red blood cell aggregation (RBCA) in a variable way when administered to hypertensive patients with low- and high-shear WBV.[24] Another study demonstrated a positive correlation between intravenous furosemide infusion and RBCA elevation.[25] Adverse HR parameters contribute to HT, but it is also hypothesized that development of microvascular complications such as hypertensive retinopathy may be partly secondary to disordered HR functions.[26] Alpha 1 inhibitor drug therapy has been shown to positively impact HR variables such as WBV, PV, and the fibrinogen level.[27] Venotonic drugs such as ruscus extract and diosmine have been studied to affect leukocyte endothelial interaction and histamine-mediated increase in vascular permeability in the animal model.[27] Probucol has been shown to have positive HR effects on patients with diabetic retinopathy apart from favorable alteration in lipid profile.[28]

A randomized double-blinded study has demonstrated that treatment with enalapril or losartan significantly reduces the mean blood pressure from pretreatment values. However, there was no statistically significant changes in the levels of hemostatic markers such as von Willebrand factor, fibrinogen, soluble P-selectin, and plasminogen activator inhibitor-1.[29] Endocan is a novel marker of endothelial dysfunction in hypertensive subjects. Treatment with amlodipine has been shown to reduce serum endocan and CRP levels, thereby demonstrating anti-inflammatory properties of amlodipine.[30]

An earlier study concluded that a 10.99% increase of Hct produced an increase of one unit relative viscosity, which means approximately a 20% increase in WBV (assuming WBV = 5 cp) for a healthy individual.[31] For the physiologic compensation of 20% increased viscosity, blood pressure increase will be 20% or vasodilation will be 4.66% in radius.[22] Our study shows that 11% reduction in Hct has resulted in 12.94% reduction in WBVh. Thus, this decrease in blood viscosity would result in appreciable reduction in blood pressure. At higher and/or chronic dosing, the contribution of improvement in HR behavior to reduction in BP is expected to be more pronounced.

The association of edema with amlodipine therapy, especially as a dose-related side effect, could also be understood in the context of hemodilution caused by the drug. As shown in this study, the effects of hemodilution are visible even at drug concentration as low as 1.39 ng/ml. Hence, at about 10 times higher drug concentrations (commonly encountered in patients with HT on long-term amlodipine therapy), the accumulation of fluid in the blood would be much higher. The body would attempt to remove this accumulated fluid through renal excretion.

Any imbalance in the kinetics of fluid accumulation and excretion would result in peripheral edema. Another possible mechanism might be hemodilution leading to decrease in intravascular oncotic pressure due to increased compliance of the vascular system compared with the extravascular space, which leads to shifting of fluid to extravascular space, causing edema. Apart from hemodilution, amlodipine was also found to increase the RBC deformability, which can enhance its antihypertensive effect.[10]

The strongly opposite behavior of RCA and RCR compared with other HR parameters with respect to the drug concentration can be understood by classifying the HR parameters as plasma related (WBV, PV, and Hct along with the hematological parameters such as WBC, RBC, and plasma hemoglobin) and RBC related (RCR and RCA). As plasma-related parameters are directly affected by hemodilution, they contribute to the reduction in WBV caused by amlodipine. The difference in the response of plasma-related and RBC-related parameters to amlodipine might result from the preferential partitioning of the drug to plasma rather than to the RBCs, although studies to substantiate this hypothesis have not be reported so far.

Thus, this study shows that administration a single dose of 5 mg of amlodipine could produce statistically significant changes in plasma-related HR parameters such as WBV at different shear rates, PV, and Hct in normal human volunteers, while the RBC-related parameters were found to be unaffected. It is more likely that in hypertensive subjects also, amlodipine would alter HR parameters significantly as shown in other studies.[10] Hence, our study results could be extrapolated to hypertensive subjects.

While hemodilution has been suggested as the main mechanism for this process, the negative correlation between the drug concentration and WBV parameters corrected for Hct suggests that an additional mechanism may also be involved. We also suggest that the antihypertensive action of amlodipine may partly be contributed by improvement in its HR properties. The hypothesis of hemodilution governing the HR behavior and possibly antihypertensive action of amlodipine and of the possible preferential partitioning of amlodipine to plasma rather than to erythrocytes need to be validated through independent studies.

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Conflicts of interest

All authors have none to declare.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ihj.2018.10.417.

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