Hemorheological effects of amlodipine in spontaneously hypertensive rats

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Abstract:
OBJECTIVES: The effect of course administration of amlodipine on whole blood viscosity and on macro- and microrheological parameters was evaluated.

MATERIALS AND METHODS: SHRs were treated intragastrically with amlodipine at a dose of 10 mg/kg for 6 weeks. After finishing the course, hemodynamic and hemorheological parameters were measured.

RESULTS: The antihypertensive treatment with amlodipine resulted in a significant decrease in mean blood pressure by 29% and left ventricular to body weight mass index by 7%. Nevertheless, BV tended to increase. The administration of amlodipine had no effect on PV, plasma fibrinogen concentration, RBC aggregation, and RBC deformability, but hematocrit was higher (by 6%) than it was in control group.

CONCLUSIONS: These results demonstrate that amlodipine has no positive hemorheological improvements when administered to SHRs.

Keywords: Amlodipine, arterial hypertension, hemorheology, spontaneously hypertensive rats

Introduction

Numerous studies have been shown that an increase in blood viscosity (BV) in patients with arterial hypertension (HT) positively correlates with a frequency of cardiovascular accidents.[1‑3] Hyperviscosity of the blood develops through the deteriorations of its macro- and micro-rheological parameters.[4] It was shown that hemorheological parameters depend on the severity of the disease and their correction leads to improving microcirculation and tissue oxygenation.[5,6] Therefore, during the treatment and investigation of the action mechanisms of a new antihypertensive drug, special attention should be paid to assessment of the effect on blood rheology.[7,8]

Calcium channel blockers (CCBs) are widely used for the treatment of HT and mechanisms of their hemodynamic effect are well known.[9] However, the data on the effect reports of CCBs on blood rheology parameters are contradictory. Numerous clinical studies showed that a decrease in BP with long-term use of CCBs (amlodipine, nicardipine, nitrendipine, and felodipine) was not followed by changes in BV in patients with HT or even tended to increase it.[10‑12] Other studies revealed that antihypertensive therapy with amlodipine resulted in a decrease in BV.[13,14] Those contradictions are difficult to explain because too little is known so far about the effect of CCBs, and particularly amlodipine, on macro- and micro-rheological parameters that determine BV.

Thus, the present study was planned to assess the effect of long-term amlodipine administration on BV as and on macro- and micro-rheological parameters in hypertensive rats.

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Materials and Methods

Animals
The experiments were carried out on 17-week normotensive Wistar-Kyoto (WKY) rats and spontaneously hypertensive rats (SHRs). This study was approved by the local bioethics committee for animal studies (Approval No. 72052014).

Drugs, kits, and chemicals
Sodium citrate (Khimprom, Russia), a reagent kit for determining concentration of fibrinogen in blood plasma «Fibrinogen-Test» (Tehnologia-Standart, Russia), thiopental sodium (Sintez, Russia), and amlodipine (Vertex, Russia) were used in this study.

Experimental design
SHRs from the experimental groups were treated daily intragastrically with amlodipine (SHRs + amlodipine) in 1% starch mucilage at a dose of 10 mg/kg for 6 weeks. The rats in both WKY and control SHRs groups were received 1% starch mucilage according to the same scheme.

Measurement of arterial blood pressure
Blood pressure (BP) was recorded with a high-throughput MP150 Data Acquisition and Analysis System equipped with a NIBP200A Automated Noninvasive Tail BP Monitoring System (Biopac Systems, Inc., USA) and AcqKnowledge 4.2 software.

Measurement of hemorheological parameters
Hemorheological measurements were carried out essentially as described previously. Briefly, blood samples were stabilized with 3.8% sodium citrate in a ratio of 9:1. Plasma fibrinogen concentration was evaluated with standard test kits on a KG-4 Coagulometer (Cormay, Poland). The hematocrit was measured by centrifugation of the whole blood in glass capillary tubes (650 g, 15 min) and expressed in vol%. BV and plasma viscosity (PV) were measured at 36°C on a rotational viscometer (LVDV-II + Pro, CP40, Brookfield Engineering Labs, USA). BV was determined at shear rate ranging 15–450 s\(^{-1}\) and PV was assessed at 450 s\(^{-1}\).

Measurement of left ventricular mass index
The animals were euthanized in a CO\(_2\) chamber. After the euthanasia, the animal’s body mass was measured. An incision was made in the chest; the heart was quickly taken out. The left ventricle (LV) was cutoff and weighted. LV mass index was calculated as the relation of LV mass to body weight (BW).

Statistical analysis
The data were analyzed statistically using Statistica 6.0 software (StatSoft, USA) as well as nonparametrical Kruskal-Wallis and Mann–Whitney tests. The results are summarized as mean ± standard error of the mean.

Results
By the end of amlodipine administration, the values of BP and LV mass index of SHRs from the control group were significantly (by 36% and 55%, respectively) higher than the values in WKY rats [Figure 1 and Table 1]. The body mass values of the animals from the three groups did not differ.

BV in control SHRs at shear rates from 15 to 450 s\(^{-1}\) was significantly higher (by 15%–19%) than in WKY rats [Figure 2]. Hematocrit and fibrinogen concentrations were also increased (by 4% and 11%, respectively) [Table 1]. Measurements of microrheological parameters showed that T\(_{1/2}\) in SHRs was lower by 20% compared to WKY, which indicates an increasing RBC aggregation [Table 1]. Elongation index did not differ at shear stress 1 Pa and was significantly lower by 2% at shear stress 20 Pa, which indicates a decreasing RBC deformability, in SHRs compared to WKY rats [Table 1].

In SHRs + amlodipine group, BP level was lower by 29% (\(P < 0.05\)) than in SHRs of the control group and did not differ from the parameter value in WKY rats [Figure 1]. LV/BW ratio in rats from the experimental group was significantly lower (by 7%) than in control group [Table 1].

After 6 weeks of amlodipine administration, BV tended to increase at shear rates from 15 to 150 s\(^{-1}\), but those differences did not reach a significant level [Figure 2]. In this group, a significant increase (by 6%) in hematocrit compared to the parameter value of SHRs from the control group was revealed [Table 1]. The administration of amlodipine had no effect on PV,

![Figure 1: The influence of course (6 weeks) administration of amlodipine (10 mg/kg) on the mean arterial blood pressure in spontaneously hypertensive rats. Each column represents the mean ± standard error of the mean (n = 10). *P < 0.05 and **P < 0.05 as compared to the Wistar-Kyoto and control spontaneously hypertensive rat group, respectively (Mann–Whitney U-test)](image-url)
Table 1: The influence of course (6 weeks) administration of amlodipine (10 mg/kg) on the left ventricular to body weight mass index, macro- and micro-rheological parameters in spontaneously hypertensive rats

| Group          | LV/BW (mg/g) | Hematocrit (%) | PV (mPa/s) | Fb (g/L) | T₁/₂ (s) | El₁5 (Pa) | El₂₀ (Pa) |
|----------------|--------------|----------------|------------|----------|----------|-----------|-----------|
| WKY            | 2.02±0.05    | 46±1           | 1.04±0.01  | 1.87±0.09| 21.1±1.2 | 0.207±0.004| 0.531±0.006|
| SHRs           | 3.12±0.06*   | 48±1*          | 1.08±0.01* | 2.13±0.08*| 16.9±0.7*| 0.210±0.006| 0.522±0.002* |
| SHRs + amlodipine | 2.89±0.05** | 51±1**        | 1.09±0.01* | 2.26±0.11*| 16.3±0.8*| 0.202±0.003| 0.517±0.002**|

Each value represents the mean± standard error of the mean (n = 10). *P<0.05 and **P<0.01 as compared to the WKY and control SHR group, respectively (Mann-Whitney U-test).

plasma fibrinogen concentration, RBC aggregation, and RBC deformability [Table 1].

Discussion

Numerous studies revealed changes in major hemorheological parameters in HT.\(^{[1,4]}\) The observed changes in blood rheology allow us to suggest that in those patients, there was a development of hyperviscosity syndrome, which is characterized by an increase in PV and hematocrit, as well as by impairment of the microrheological properties of RBC: increase in their aggregation and decrease in deformability. However, it is still questionable whether this syndrome could be corrected with antihypertensive drugs.

Currently available information about the effect of amlodipine on various hemorheological parameters in HT is rare and contradictory. In some clinical research papers, it was shown that amlodipine had no considerable effect on blood rheology.\(^{[10-12]}\) For example, Kearney-Schwartz et al. showed that after 2-month therapy with amlodipine at a dose of 5 mg/kg in patients with arterial HT complicated by type 2 diabetes mellitus, PV tended to increase with no changes in PV, hematocrit, and RBC aggregation.\(^{[10]}\) In another investigation, it was shown that no significant changes in all studied hemorheological parameters (BV, hematocrit, RBC aggregation, and RBC deformability) were noted in hypertensive patients after 3-month amlodipine therapy at doses of 5~10 mg/kg.\(^{[12]}\) In the study of Aksnes et al., after amlodipine treatment at a dose of 10 mg/kg for 2 months, a significant decrease in hematocrit in HT was observed, with just a tendency of BV to decrease at lower shear rates.\(^{[14]}\) On the other hand, Linde et al. showed that after 4-month amlodipine therapy in patients with previously unattended HT, positive changes in some hemorheological parameters were observed: BV at lower shear rates and PV decreased, RBC deformability increased.\(^{[13]}\) The analysis of clinical studies of amlodipine indicates that the difference in the results could be associated with different doses of the drug and duration of its administration, different stages of HT in the patients, and presence or absence of previous treatment. Thus, in our view, the use of inbred animals is more reasonable for understanding of the basic effects of amlodipine on the rheological parameters of blood.

SHR is a commonly accepted model of HT, where high BP values are generally caused by increased total peripheral resistance (TPR) and are not associated with other pathologic processes.\(^{[15]}\) Blood hyperviscosity syndrome in SHRs is characterized by increased BV and PV, high hematocrit values, enhanced RBC aggregation, and decreased RBC deformability.\(^{[16]}\)

In our experiment, when amlodipine was administered to SHRs, a pronounced therapeutic effect of the drug on hemodynamic parameters was revealed: reduction in BP to the values of normotensive animals and decrease of LV hypertrophy. However, the study results showed that amlodipine has no effect on BV when administered to SHRs. Furthermore, at lower shear rates, even the tendency of the BV to be increased compared to control hypertensive rats was noted. As changes of BV depend on changes in macro- and micro-rheological parameters, it was important to study the effect of the drugs on each of these parameters with the analysis of possible contribution of these rheological alterations to changes in BV.

It was shown that increase in hematocrit is an independent risk factor for the development of HT complications.\(^{[3]}\) In SHRs, an overt increased of hematocrit is reported, which was observed also in this study. Stable increase in the ratio of RBC volume for different forms of HT can be caused by erythrocytosis and/or plasma volume reduction.
In our study, the administration of amlodipine resulted in even greater hematocrit increase in SHRs. Similar results were also obtained in another study performed on hypertensive animals. In the literature, there is no information about changes in RBC quantity or volume in SHRs under the influence of amlodipine. On the other hand, it is well known that amlodipine has a slight diuretic effect, which may cause a reduction in plasma volume.

PV is another macrorheological factor that significantly contributes to BV. Generally, an increase in PV in cases of HT is associated with higher concentration of plasma proteins, with fibrinogen being the main protein that directly affects PV. In this study, a significant increase in PV in SHRs compared to normotensive animals was revealed. The use of amlodipine did not cause PV to fall in SHRs, which can be explained in part by the lack of effect on plasma fibrinogen concentration.

In our study, there is no change in RBC aggregation in SHRs after amlodipine administration. It should be noted that even in those studies, where positive effects of amlodipine on the rheological parameters of blood were revealed, no improvement in RBC aggregation was found. Thus, in vitro studies are in needed to reveal the direct influence of amlodipine on this parameter to exclude hematocrit and fibrinogen level factors.

In many pathological conditions, including various forms of HT, a decrease in the viscoelastic properties of the erythrocyte membrane is observed. A number of studies showed an increase in erythrocyte membrane rigidity as HT progressed. Lowered erythrocyte deformability in cases of different forms of HT significantly reduces the possibility of the passage of erythrocytes through the capillaries, leading to disruption of the microcirculation and impaired tissue oxygenation.

In our study, only slight change in erythrocyte deformability in SHRs was found. Administration of amlodipine did not have any significant effect on this microrheological parameter. In some authors’ opinions, the impact of CCBs on RBC deformability may vary depending on the degree of initial rigidity of RBC.

Conclusion

The study results demonstrate the lack of effect of amlodipine on BV when administered to SHRs. Despite a significant improvement in hemodynamic parameters (a decrease in BP as low as WHY level, reduction of myocardial hypertrophy), BV tended to increase which is due to increase in hematocrit in the setting of lack of amlodipine effect on other hemorheological parameters. Consequently, with amlodipine as an antihypertensive drug, the effect on BV as an additional reserve of TPR reduction remains unexhausted.

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Conflicts of interest

There are no conflicts of interest.

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