Mesenchymal Stem Cells From Adipose Tissue Do not Improve Functional Recovery After Ischemic Stroke in Hypertensive Rats

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Background and Purpose—Hypertension is the most frequent comorbidity in stroke.The purpose of this study was to evaluate whether hypertension alters the response to treatment with adipose tissue-derived mesenchymal stem cells (ADMSCs) after an ischemic stroke in rats.

Methods—Ischemic stroke was induced in male normotensive or hypertensive rats. Either vehicle or 1×10^6 ADMSC was intravenously administered at 48 hours poststroke. Functional outcome, lesion size and volume, and markers of brain repair (GFAP [glial fibrillary acidic protein], doublecortin, CD-31, α-smooth muscle actin) were evaluated.

Results—Hypertensive rats had larger lesions, higher apparent diffusion coefficients (ADC) and worse functional outcomes than normotensive rats. Hypertension increased GFAP and vascular markers (CD-31 and α-smooth muscle actin). The hypertensive rats treated with ADMSC did not show any significant improvement in functional recovery, lesion size, ADC values, or histological markers compared with those which received the vehicle.

Conclusions—ADMSC did not reverse the hypertension-induced increase in lesion severity or functional impairment. Gliosis, neurogenesis, or vascular markers were not affected by ADMSC in hypertensive rats. Hypertension has a negative impact on the therapeutic effect of ADMSC after an ischemic stroke.

Visual Overview—An online visual overview is available for this article. **(*Stroke*. 2020;51:342-346. DOI: 10.1161/STROKEAHA.119.027133.)**

Key Words: brain ■ comorbidity ■ hypertension ■ neurogenesis ■ stem cells

Hypertension is considered one of the most common and important vascular risk factors for stroke, affecting >80% of those patients.[1]

Cell therapy is a promising treatment for stroke, and studies have shown that the administration of adipose tissue-derived mesenchymal stem cells (ADMSC) can lead to improved functional recovery and brain repair after a stroke.[2]

Because of the high percentage of patients with comorbidities such as hypertension, stroke research recommendations consider comorbidities to be a key element to be studied to successfully translate the experimental data to the clinic.[3,4]

The aim of this study was to assess the impact of hypertension on stroke in rats. It also evaluated the effect of hypertension on the therapeutic response to the intravenous administration of human ADMSC (hADMSC) in an experimental stroke model.

Methods

The original data are available from the author for correspondence upon request.

Ethics Statement

Animal care and experimental procedures were performed in strict compliance with the Guide for the Care and Use of Laboratory

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Animals, and the study was approved by La Paz University Hospital's Ethics Committee, according to the Spanish and European Union rules (86/609/CEE and RD53/2013). Experiments were conducted according to the stroke therapy academic industry roundtable[3] and ARRIVE (Animal Research: Reporting of In Vivo Experiments)[5] guidelines in terms of randomization, blinding, and statistical power.

Animals, Hypertension, and Surgery

These experiments were conducted on adult (9–10 weeks old) male spontaneously hypertensive rats (SHR) and normotensive Wistar rats weighing 200 to 250 g. The rats were randomly divided into 5 groups of 10 rats each: (1) sham group: rats subjected to surgery without infarction; (2) vehicle-normotensive group: normotensive rats subjected to a permanent middle cerebral artery occlusion and vehicle; (3) vehicle-hypertensive group: SHR subjected to a permanent middle cerebral artery occlusion and vehicle; (4) hADMSC-normotensive group: normotensive rats subjected to a permanent middle cerebral artery occlusion and hADMSC administration; and (5) hADMSC-hypertensive group: SHR subjected to a permanent middle cerebral artery occlusion and hADMSC administration. Vehicle or 1×10^6 hADMSC in 1 mL of 0.9% NaCl was administered via the tail vein 48 hours after surgery. Rats were euthanized for histology at 6 weeks poststroke (Figure 1). See the Methods section in the online-only Data Supplement for more information.

Results

Hypertension-Induced Impairments in Motor and Sensory Functions Which Could Not Be Reversed With hADMSC Treatment

See the Results section in the online-only Data Supplement for more information.

The vehicle-hypertensive group exhibited more severe neurological deficits compared with the vehicle-normotensive group as measured by the beam-walking test at 7 days (P=0.031), 3 weeks (P=0.014), and 6 weeks (P=0.001) and by the adhesive-removal test at 7 days (P=0.034). hADMSC resulted in a significant improvement in normotensive animals compared with their vehicle in Rogers test at 3 weeks (P=0.011) and 6 weeks (P=0.0001; Figure 2A and 2B), hADMSC-hypertensive rats did not show an improved functional outcome compared to the vehicle-hypertensive group either in Rogers test (P=0.095), or in the beam-walking test (P=0.356) and the adhesive-removal test (P=0.408) at 6 weeks. hADMSC-normotensive rats showed significant recovery in Rogers test at 6 weeks (P=0.028), in the beam-walking test at 3 (P=0.008) and 6 weeks (P=0.043); and in the adhesive-removal test at 7 days (P=0.017) compared with hADMSC-hypertensive rats (Figure 2A and 2B).

Hypertension Increased Brain Damage and hADMSC Treatment Did Not Reduce the Infarct Size

The vehicle-hypertensive rats had larger lesions compared with the vehicle-normotensive rats at 24 hours (P=0.0001) and at 6 weeks (P=0.0001). hADMSC treatment did not decrease lesion size either in hypertensive or in normotensive rats compared with their respective vehicle groups (P=0.156 and P=0.86) after 6 weeks. hADMSC-normotensive rats had significantly smaller lesions compared with hADMSC-hypertensive rats after 6 weeks (P=0.01; Figure 2C).

After 6 weeks, the vehicle-hypertensive rats had significantly higher diffusion coefficient values compared with the vehicle-normotensive rats (P=0.0001). hADMSC-normotensive and hADMSC-hypertensive rats did not show significant differences in relative apparent diffusion coefficient values compared with their respective vehicle groups (P=0.114 and P=0.211, respectively). hADMSC-normotensive rats showed significantly lower relative apparent diffusion coefficient values compared with hADMSC-hypertensive rats after 6 weeks (P=0.0001; Figure 2C).

After 6 weeks, vehicle-hypertensive rats showed no significant differences in the number of cortical motor neurons compared with vehicle-normotensive rats (P=0.694). The hADMSC increased the number of motor neurons in the normotensive group (P=0.0001) but not in the hypertensive group (P=0.398) compared with their controls. The hADMSC-treated rats in the normotensive group showed higher numbers of motor neurons compared with hADMSC-treated rats in the hypertensive group (P=0.008; Figure 2D).

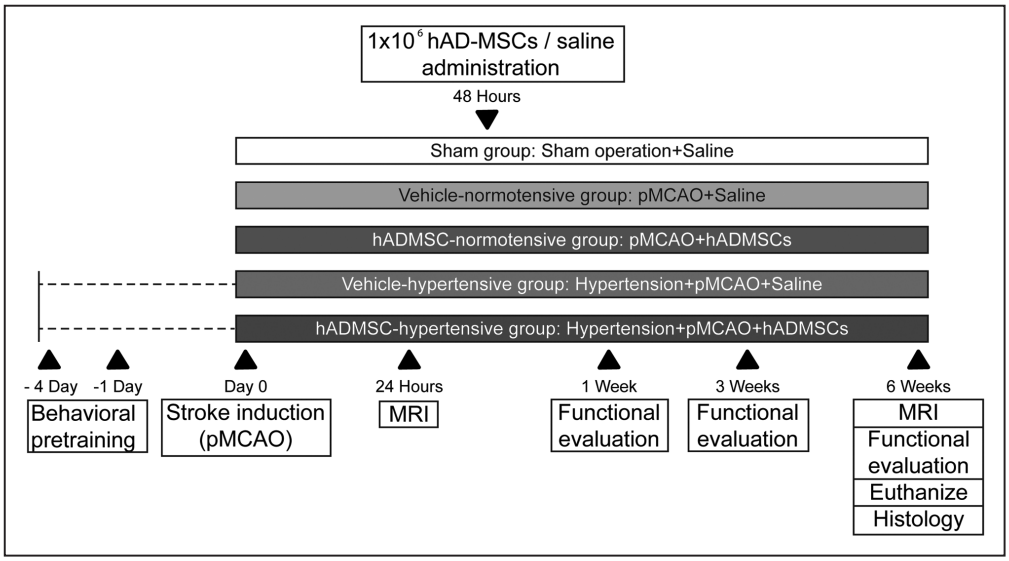

Figure 1. Study design. Ischemic stroke was induced by permanent middle cerebral artery occlusion (pMCAO). Human adipose tissue-derived mesenchymal stem cell (hADMSC) or vehicle was administered intravenously 48 h after surgery. Behavioral performance and magnetic resonance imaging (MRI) were evaluated throughout the follow-up. Animals were euthanized after 6 wk.

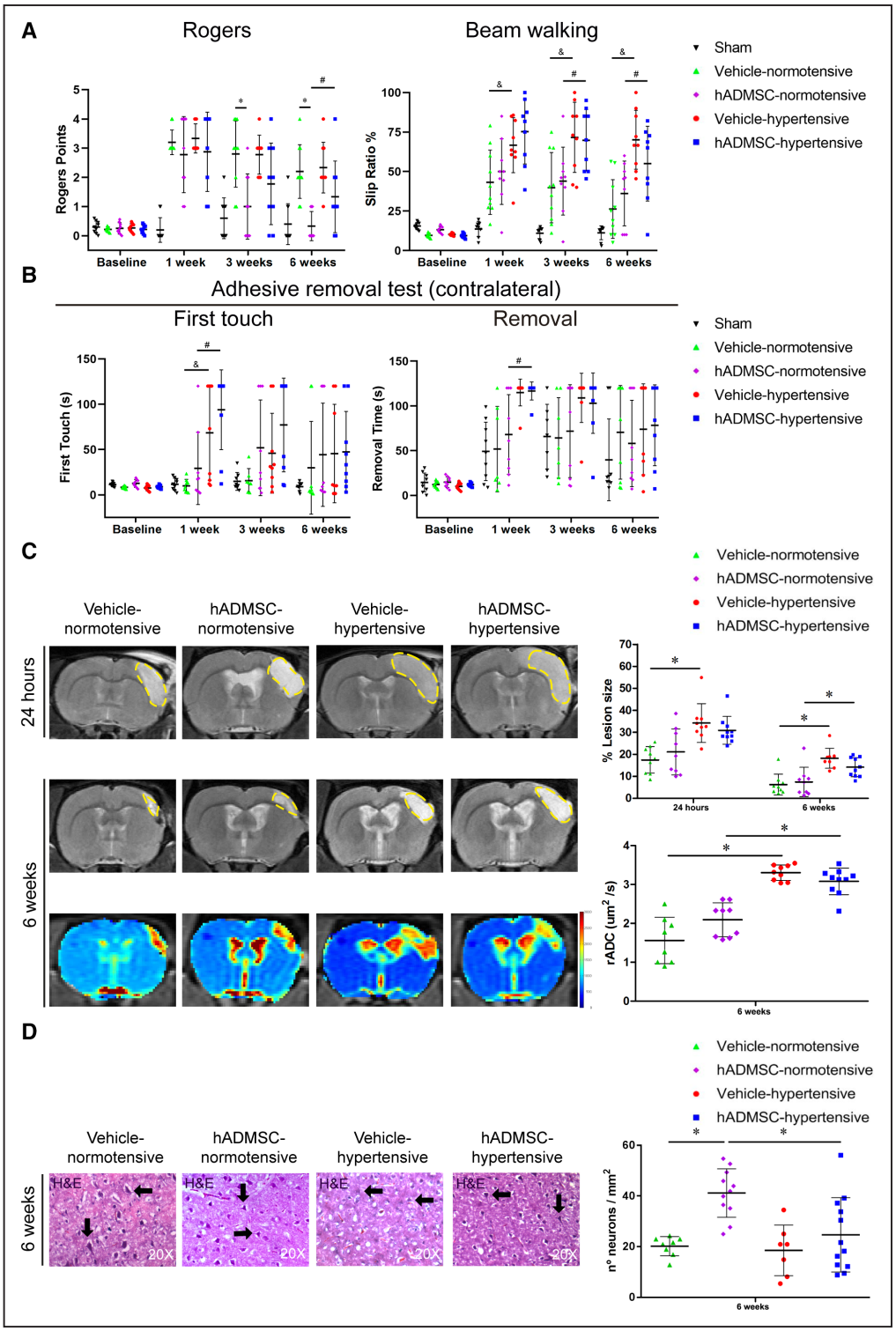

Figure 2. Behavioral tests and lesion analysis. **A** and **B**, Functional outcome. Assessment of behavioral outcome in Rogers test (**left**), walking beam (**right**) (**A**), and adhesive-removal test (**B**) at baseline and follow-up. &: vehicle-normotensive vs vehicle-hypertensive; *: vehicle-normotensive vs hADMSC-normotensive; #: hADMSC-normotensive vs hADMSC-hypertensive (n=10 rats per group). **C**, Magnetic resonance imaging (MRI) analysis. Lesion size analysis and relative apparent diffusion coefficient (rADC) measurement at 24 h and 6 wk after treatment (n=10 rats per group). **D**, Histopathologic analysis. Representative images and quantification of the motor neurons in the cortex at 6 wk after treatment (3 rats per group, 4 sections in each rat per group). Data are shown as mean±SD.*P<0.05.

Hypertension Increased Astrocyte Marker Levels and hADMSC Treatment Did Not Reverse It

There was an increase in the GFAP signal in the vehicle-hypertensive group compared with vehicle-normotensive rats (P=0.021). Treatment with hADMSC decreased GFAP signal in hADMSC-normotensive rats compared with their vehicle control (P=0.041). We did not observe any changes in the GFAP signal between vehicle- and hADMSC-hypertensive

rats (P=0.498). A significantly higher GFAP signal was found in the hADMSC-hypertensive group compared with the hADMSC-normotensive group (P=0.006; Figure 3).

Hypertension Had No Effect on Neurogenesis, and This Was Not Affected by hADMSC Treatment

We found no differences in the doublecortin signal between vehicle-hypertensive and vehicle-normotensive animals (P>0.05). hADMSC increased the doublecortin signal in normotensive rats (P=0.005) but not in hypertensive rats (P=0.631). No significant differences were found between the treatment groups (P=0.839; Figure 3).

Hypertension Increased CD-31 and α-Smooth Muscle Actin Signals and hADMSC Had No Effect on These Vascular Proteins

Increased CD-31 and α-smooth muscle actin (α-SMA) signals were found in the vehicle-hypertensive animals compared with the vehicle-normotensive group (P=0.0001).

Although treatment with hADMSC decreased the α-SMA signal in normotensive rats compared with their vehicle control (P=0.001), there were no differences in CD-31 and α-SMA signal between the hADMSC- and the vehicle-hypertensive groups (P=0.56 and P=1.00, respectively). hADMSC-hypertensive rats showed higher CD-31 and α-SMA signals than hADMSC-normotensive animals (P=0.048 and P=0.0001, respectively; Figure 3).

Discussion

hADMSC treatment has demonstrated efficacy and safety in the treatment of stroke in animal models.[2] The results of our previous studies showed that ADMSC administration increased the levels of brain repair markers associated with improved functional recovery in normotensive stroke animals.[6]

Comorbidities may exert a detrimental impact on treatment efficacy.[7] However, the influence of hypertension on the response to hADMSC treatment has not been thoroughly explored in ischemic stroke.

The present study provides evidence that hADMSC had no beneficial effects on hypertensive rats poststroke. The results showed that hypertension increased the lesion volume and rADC values, which may inhibit behavioral recovery. ADC

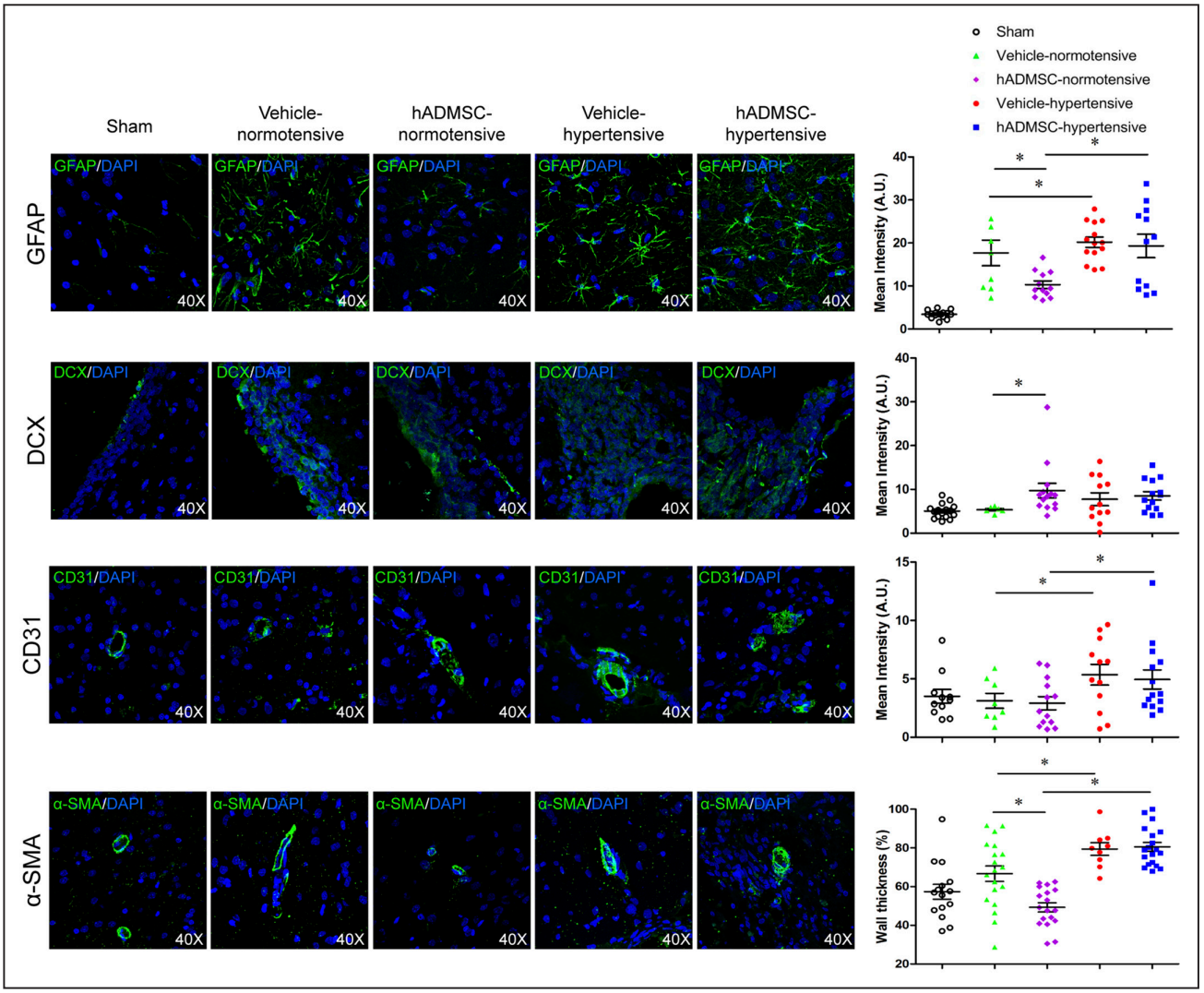

Figure 3. Representative images and quantification of GFAP (glial fibrillary acidic protein), doublecortin, CD-31, and α-smooth muscle actin (α-SMA) markers (3 rats per group, 4 sections in each rat per group). Data are shown as mean±SD.*P<0.05.

modifications could be related to the relative increase in water content of the tissue as has been reported in SHR.[8] This worse preservation of the tissue could be partly a reason why hADMSC had no beneficial effects on hypertensive stroke rats.

Our results suggest that hypertensive rats with an ischemic stroke showed increased astrocytic activation. Astrogliosis has been associated with behavioral impairment in the SHR model.[9] Treatment with hADMSC was not able to reverse astrogliosis. The exacerbation of astrogliosis mediated by hypertension may be one of the reasons for the absence of any positive effects from hADMSC treatment in hypertensive rats with ischemic stroke. After stroke, the endogenous brain repair is activated.[10] Our results demonstrated that the therapeutic modulation of neurogenesis can be limited by hypertension. Another possible explanation may be an exhausted neurogenic reserve.[11]

Conclusions

Our data show that hypertension increased lesion size and behavioral impairment in an animal model of stroke. The administration of hADMSC did not reduce lesion volume or functional deficits and had no effect on gliosis, neurogenesis, or vascular marker levels in hypertensive rats. These results suggest a negative impact of hypertension on the therapeutic effect of hADMSC after an ischemic stroke. Hypertension may be one of the reasons for the unsuccessful translation of experimental stroke therapies to the clinic.

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Disclosures

None.

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