



CEDEME - Center of Development of Experimental Models for Medicine and Biology

Regeneration of brain tissue by mesenchymal stem cells in a stroke model involves both raised nitric oxide and VEGF as decreased oxidative stress and apoptosis to healthful levels

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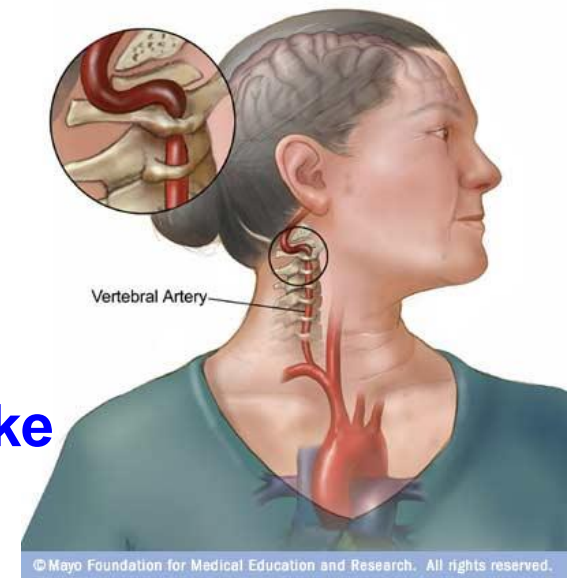
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- **Stroke or Cerebral Vascular Accident entails:**

- 10% of total deaths / World
- Interruption in blood flow: Ischemic or hemorrhagic
- Causes: hypertension, hemorrhage, blood vessel malformation, thrombus, tumor, inflammation and injuries

- **Importance of stem cell therapy for stroke is related to:**

- Differentiation potential;
- Production of bioactive substances and secretion of factors;
- Inexistence of rejection.



Initial challenges:

- Finding an appropriate model for adult stem cells therapy:
Stroke Prone Spontaneously Hypertensive Rat (SHRSP)
- Defining parameters to be measured: oxidative stress, apoptosis, neurogenesis, morphology and secreted factors
- Finding methods to track the location of stem cells in vivo:
labeling with the inside cell fluorescent dye CFSE *
- To propose a cellular therapy for spontaneous stroke:
mesenchymal SC / neural cell inducers

* CFSE – carboxyfluorescein succinimidyl ester

Stroke model: SPSHR



- SHRSP - Stroke Prone Spontaneously Hypertensive Rat
- Severe hypertension, blood pressure ~ 220 mm Hg , stroke ~ one year of age;
- Origin: Wistar → Wistar Kyoto (WK) → SHR → SPSHR
- Genetic errors in enzymes, coenzymes and cofactors that regulate blood pressure
- Deficient in tetrahydrobiopterin - BH4 - cofactor of NO synthases;
- Increased levels of superoxide in aorta, DNA damage-type 8-hydroxy guanosine, apoptotic neuronal death, lesions in the cortex (CA1 and hippocampus)
- ↓SOD in CA1 - CA3 and hippocampus, ↓NOS in cortex (Kimoto-Kinoshita, 1999);
- They are also models of genetic hypertension and elevated oxidative stress.

Animal models for cell therapy of stroke:

Control: **WKY**

For injection of SC via cisterna magna: **SHRSP**

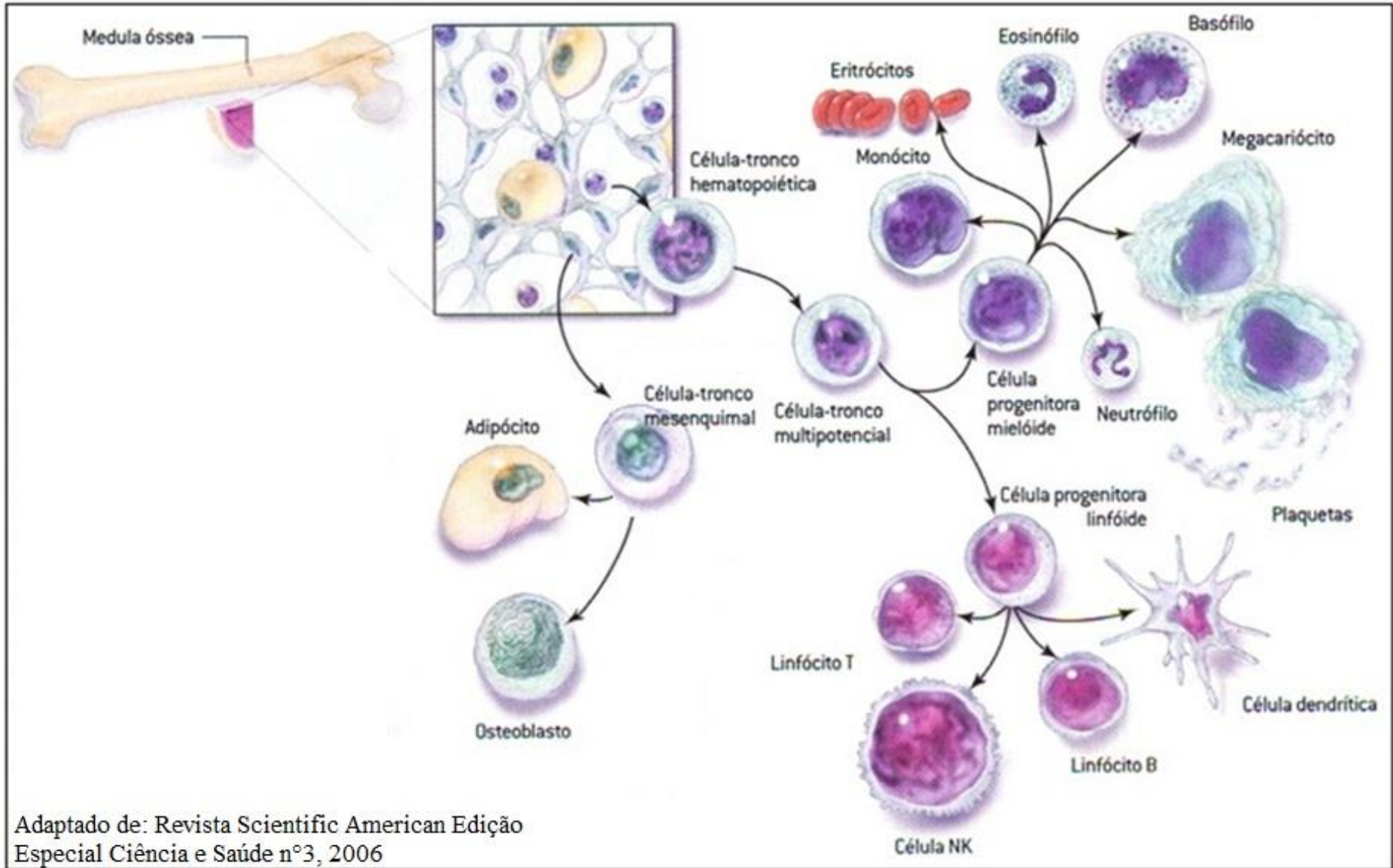
N= 10/group, age = 3 months and 1 year

Transplantation: Autologous or heterologous?

- Depending on the quality and viability of SC



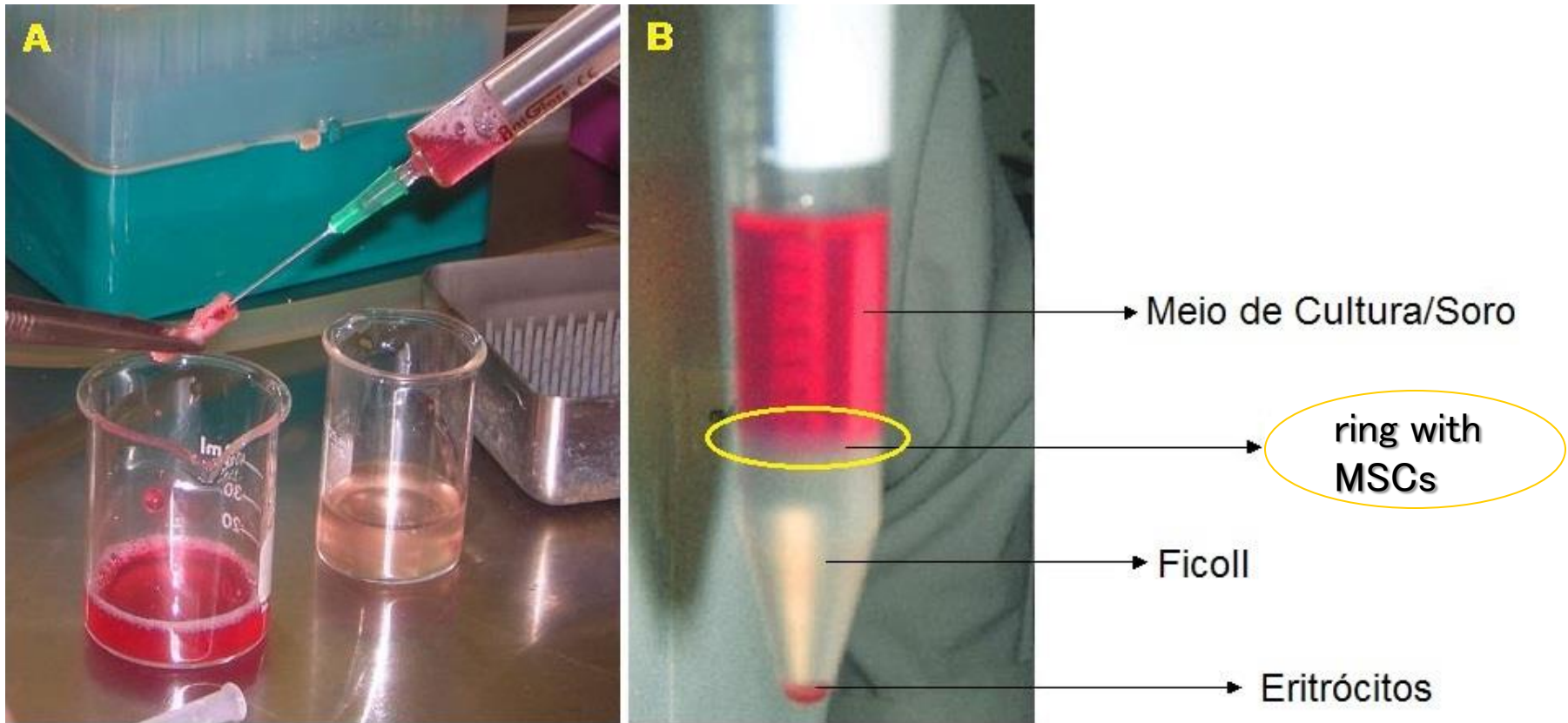
Adult stem cells from bone marrow



Adaptado de: Revista Scientific American Edição Especial Ciência e Saúde n°3, 2006

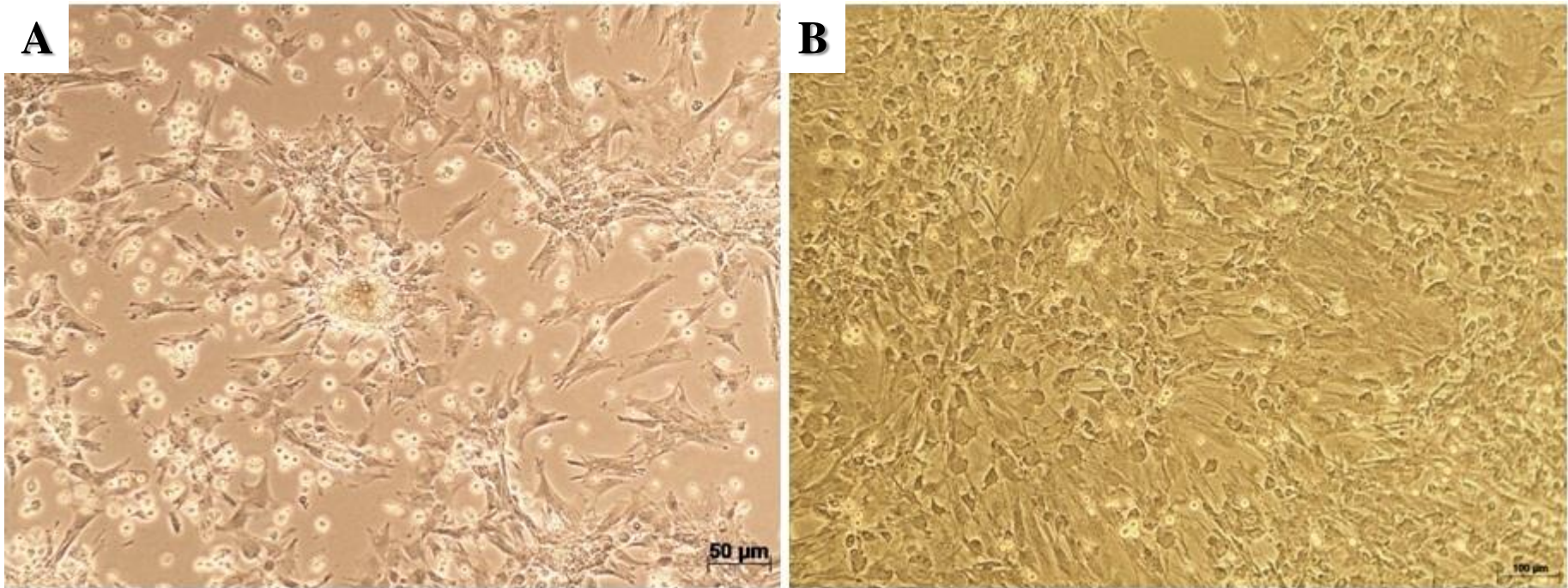
Mesenchymal stem cells from bone marrow = 0,01 a 0,001%

Isolation of Mesenchymal Stem Cells (MSCs) from Bone Marrow of Rats

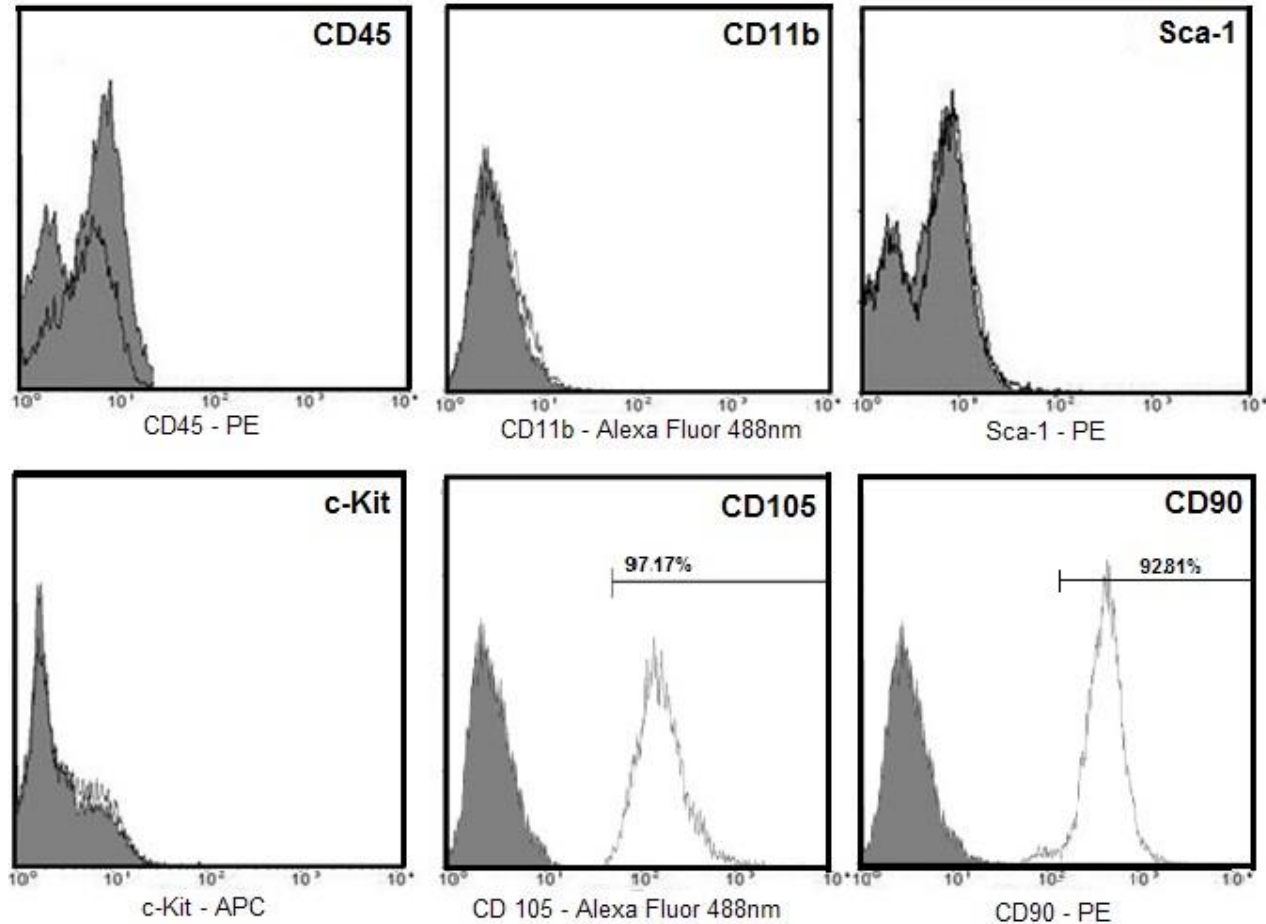


(A) Bone marrow perfusion with culture medium via femoral orifices
(B) Separation of cells by Ficoll gradient

Cultures of stem cells from the bone marrow of rats

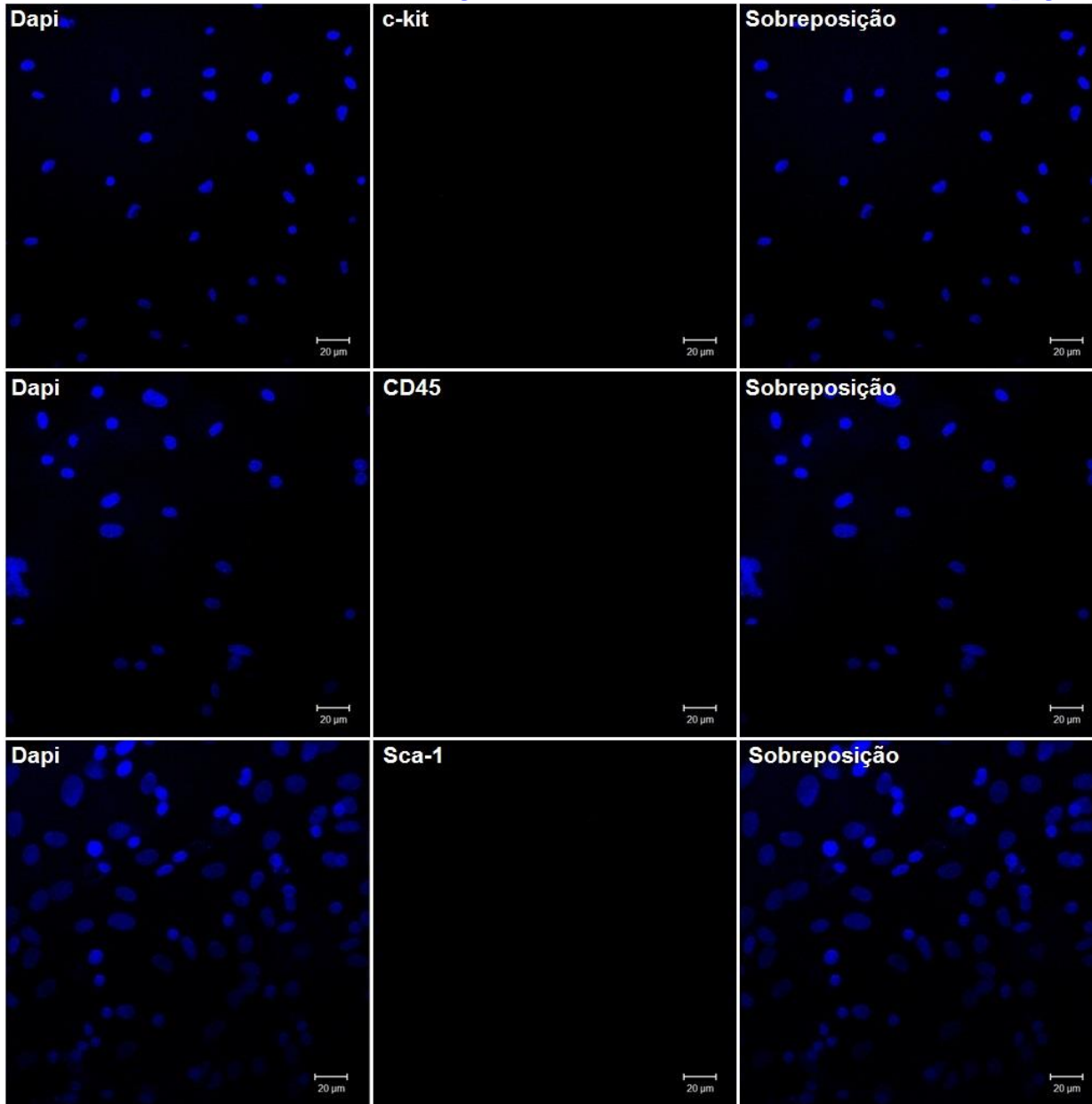


Morphology of MSC. Bone marrow MSC exhibited fibroblast-like morphology. Images were taken from cultures at 7 (A) and 14 (B) days after plating. **(A)** Note the presence of two types of cells: small, round cells (hematopoietic stem cells) and long-shaped cells (MSC). Scale = 50 μm. **(B)** At this stage, the cells displayed the typical “fibroblast-like” morphology shown here. The cells did not spontaneously differentiate during culture expansion. Scale bar = 100μm.



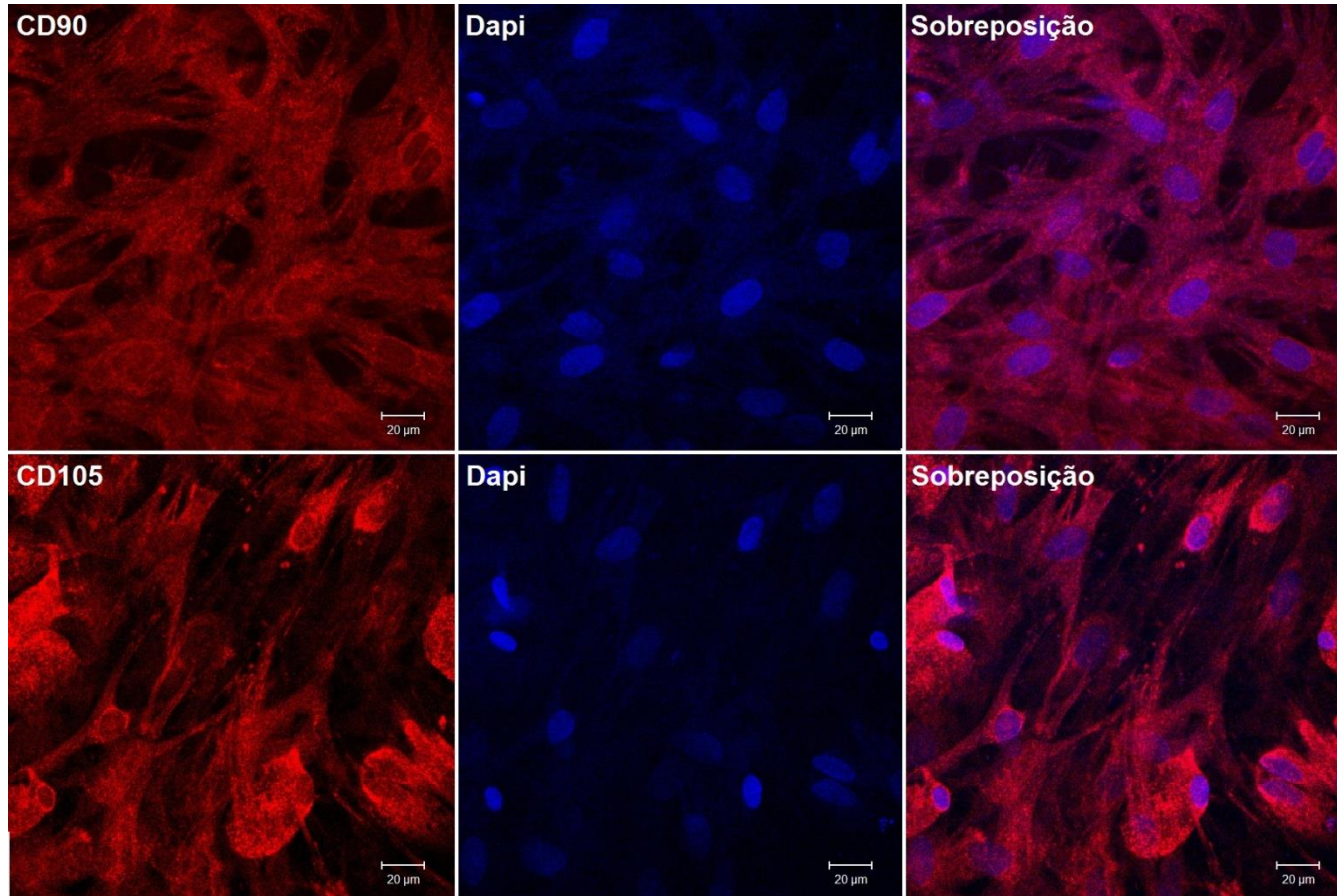
MSC characterization. Fluorescent activated cell sorter of cell-surface antigen expression The expanded attached, mesenchymal cells were positive for CD90 and CD105, which are markers of MSC. In contrast, the cells were negative for other markers of the hematopoietic lineage, including CD45, Cd11b, c-kit and Sca-1.

Characterization of mesenchymal stem cells from bone marrow of rats by confocal microscopy - negative marking

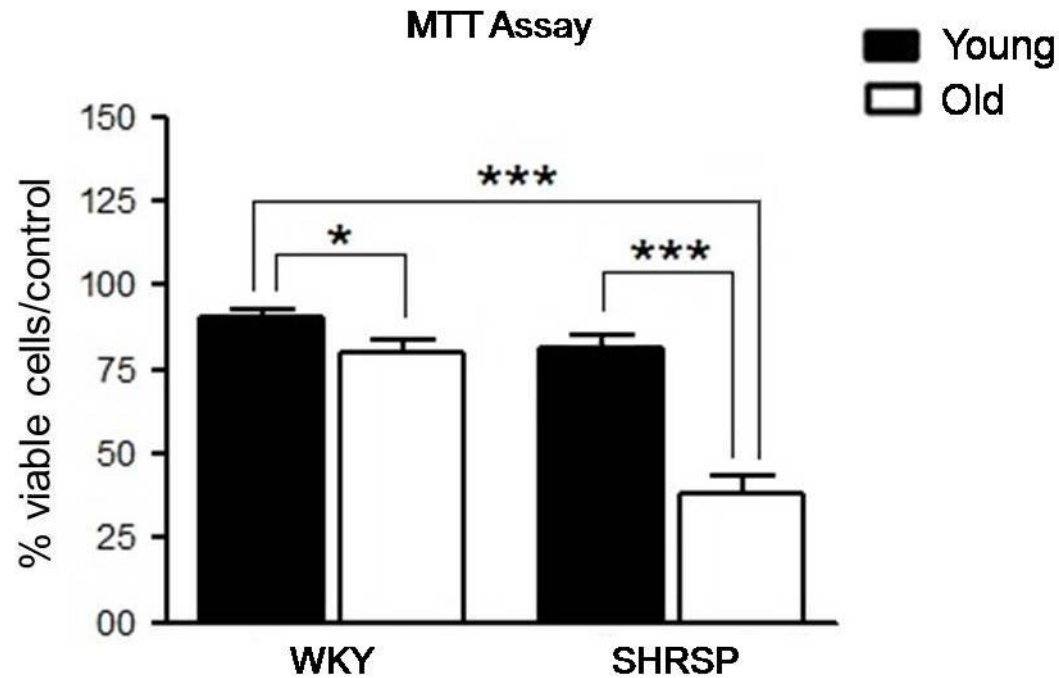


cells were negative for markers of the hematopoietic lineage, including CD45, Cd11b, c-kit and Sca-1

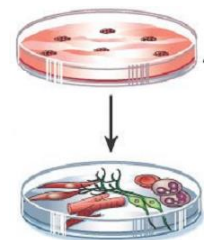
Characterization of mesenchymal stem cells from bone marrow of rats by confocal microscopy: positive staining



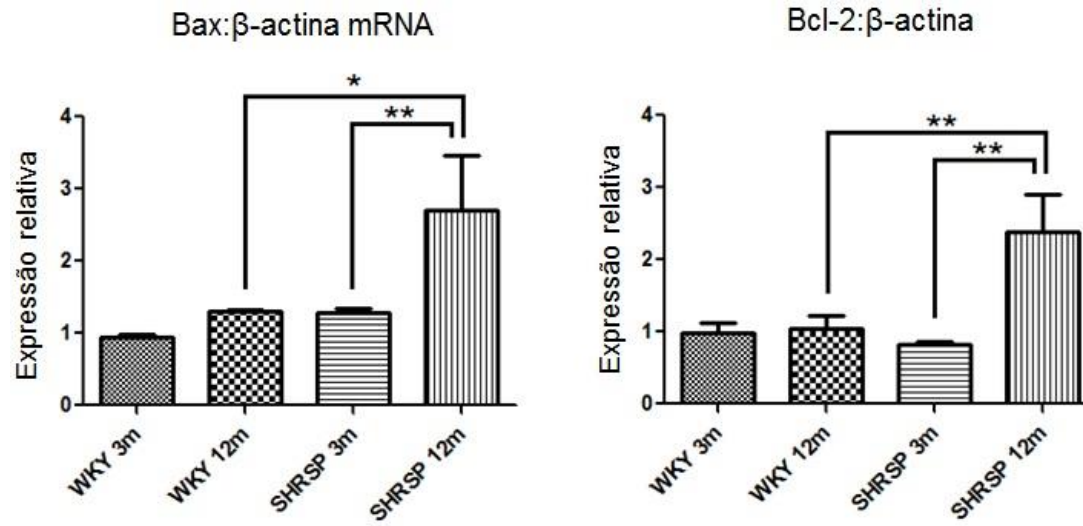
MSCs were double stained in blue with DAPI nuclear stain CD90, CD105 antigens. The bright red cells indicate positivity.
Scale bar = 20 µm.



Viability of stem cells from normotensive WKY and hypertensive SHRSP by MTT *** $p < 0,0001$.

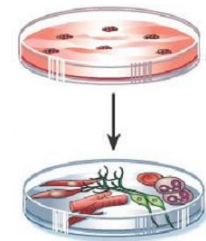


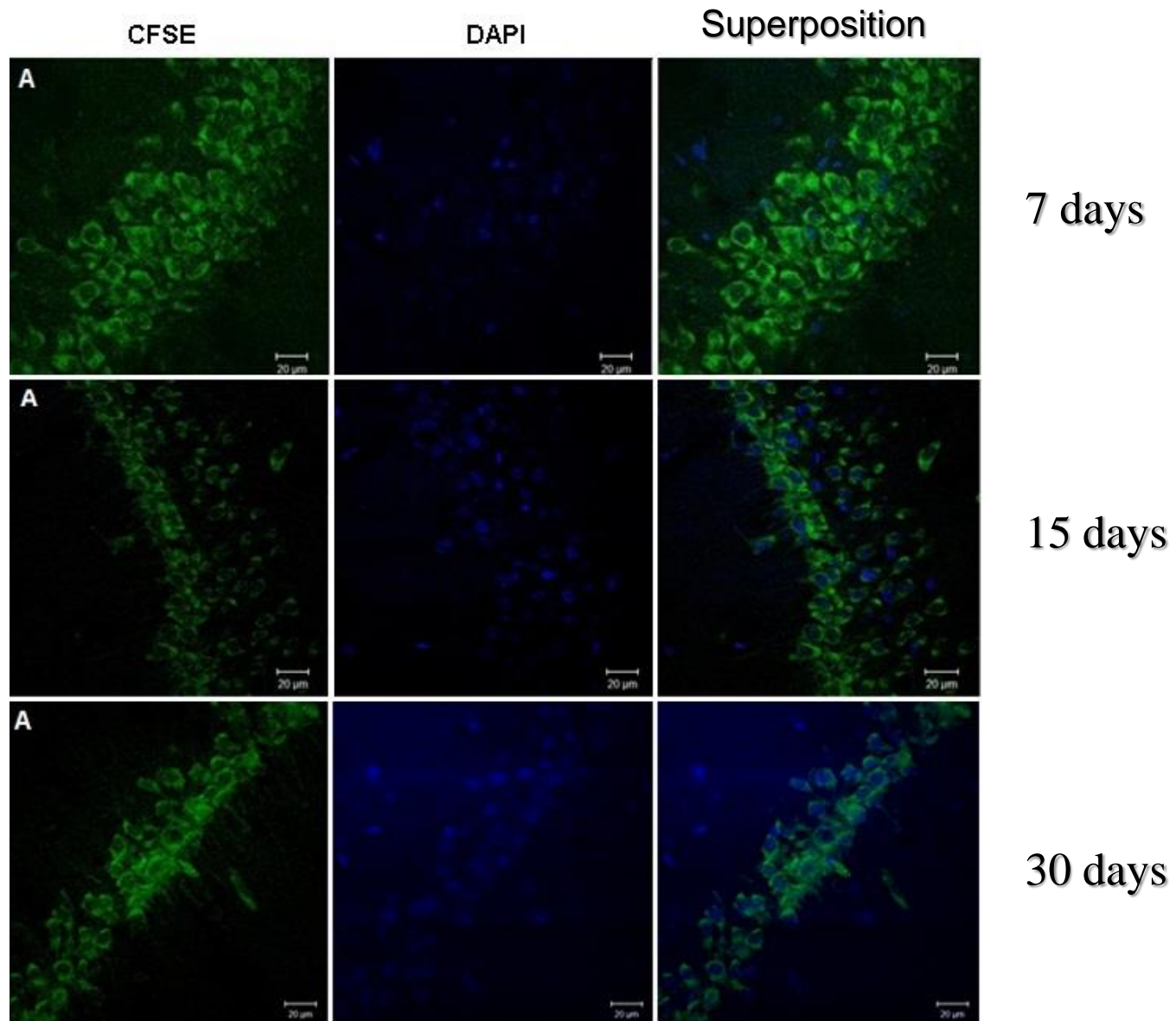
Quantitative analysis of the expression of bax and bcl-2 in mesenchymal cells culture with normal and hypertensive animals at 3 or 12 months of age



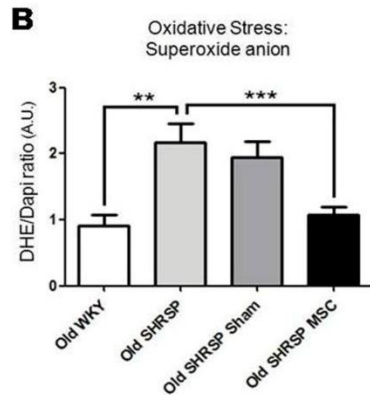
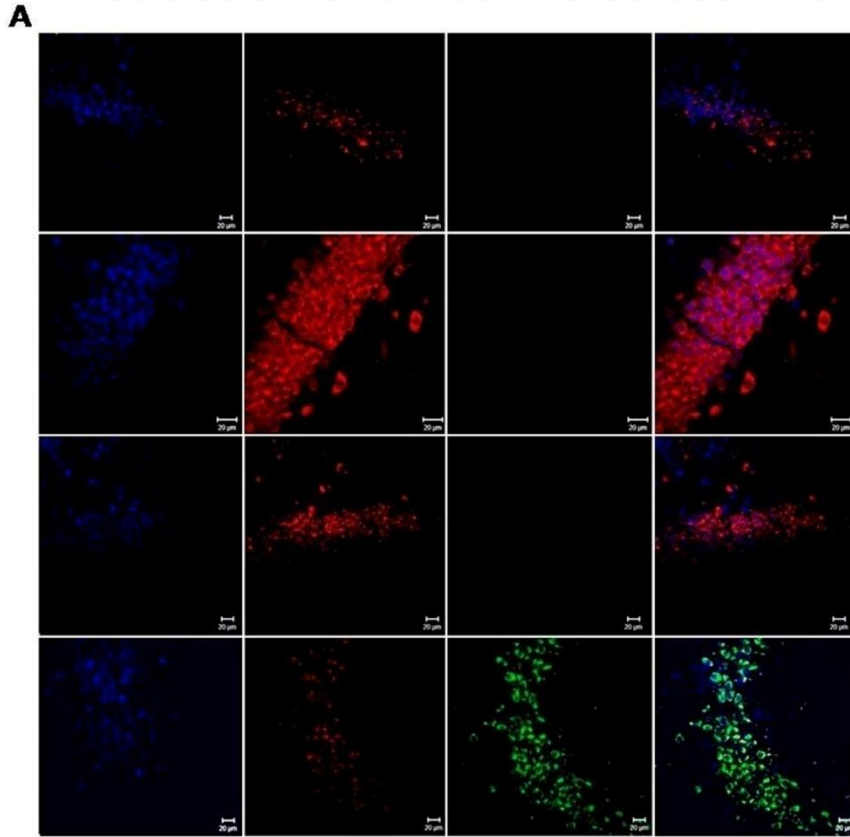
Real Time RT-PCR markers of apoptosis markers in cultured mesenchymal stem cells from WKY e SPSHR. * = $p < 0,05$; ** = $p < 0,01$. N = 10 /group.

- Bax and Bcl2 – heterodimers?
- Stem cells from young animals are more viable and less susceptible to apoptosis when derived from WKY.
- Transplantation from WKY 3 months to SHRSP 1 year old.



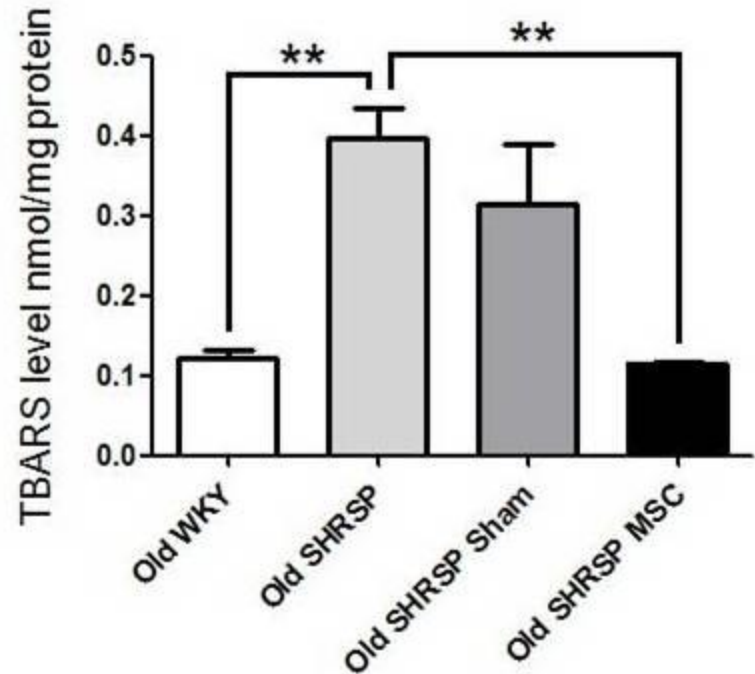


Reduction of oxidative stress in the brain after stem cells transplantation



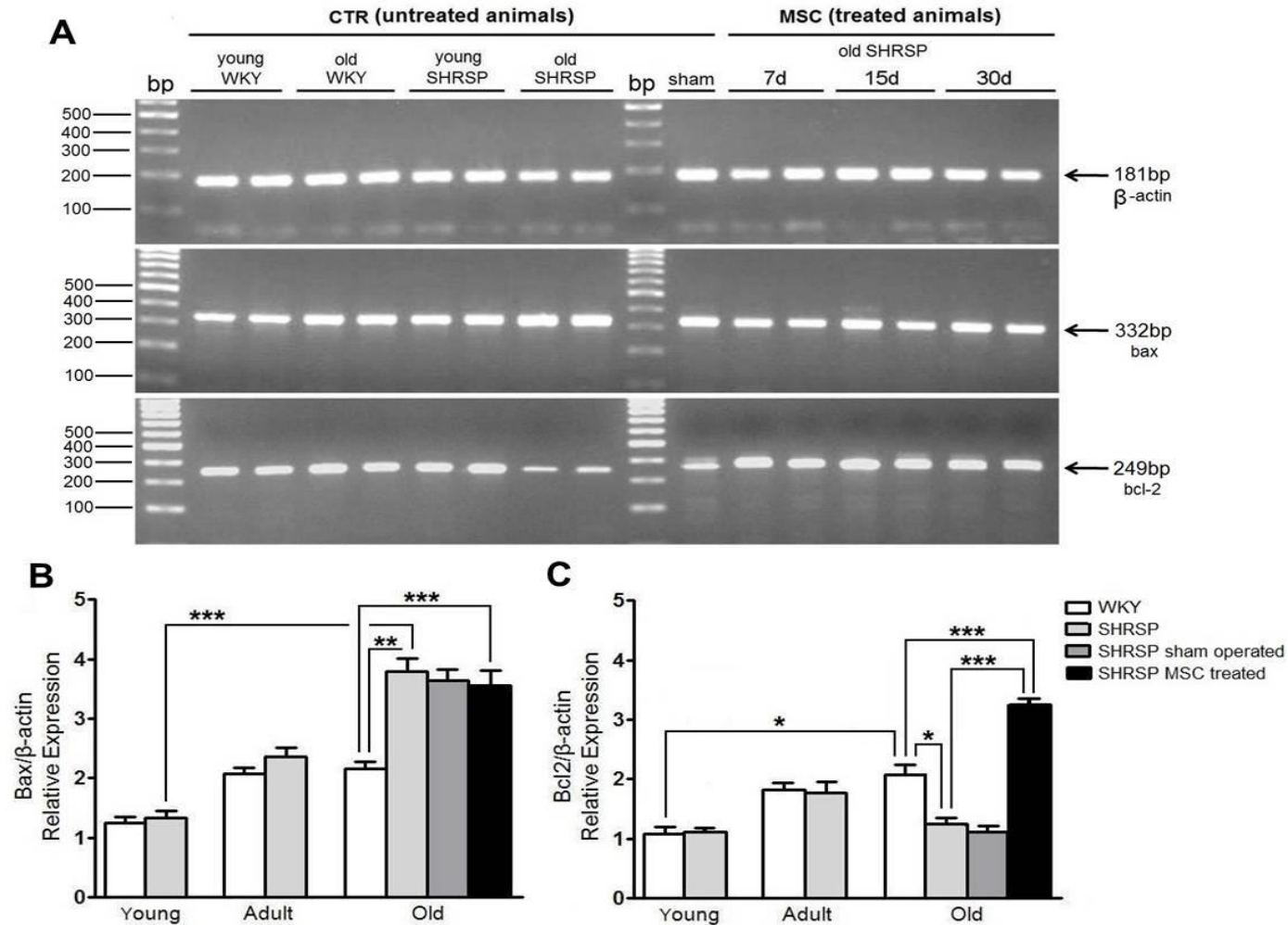
Confocal microscopy: Increased superoxide in the brain isolated from stroke-prone spontaneously hypertensive rats, compared with their normotensive controls Wistar Kyoto, as detected by Dihydroethidium, DHE – red spots and DAPI (all blue nucleus). The MSC migrated to places of greater production of superoxide anion. Note the co-localization of MSC (green) and superoxide anion (red) in treated animals. Scale bar = 20μm. **(B)** Quantification and analysis of figure pixilation. Results are expressed as mean ± SEM, represented by error bars; **p < 0.01; ***p < 0.001.

Lipid peroxidation



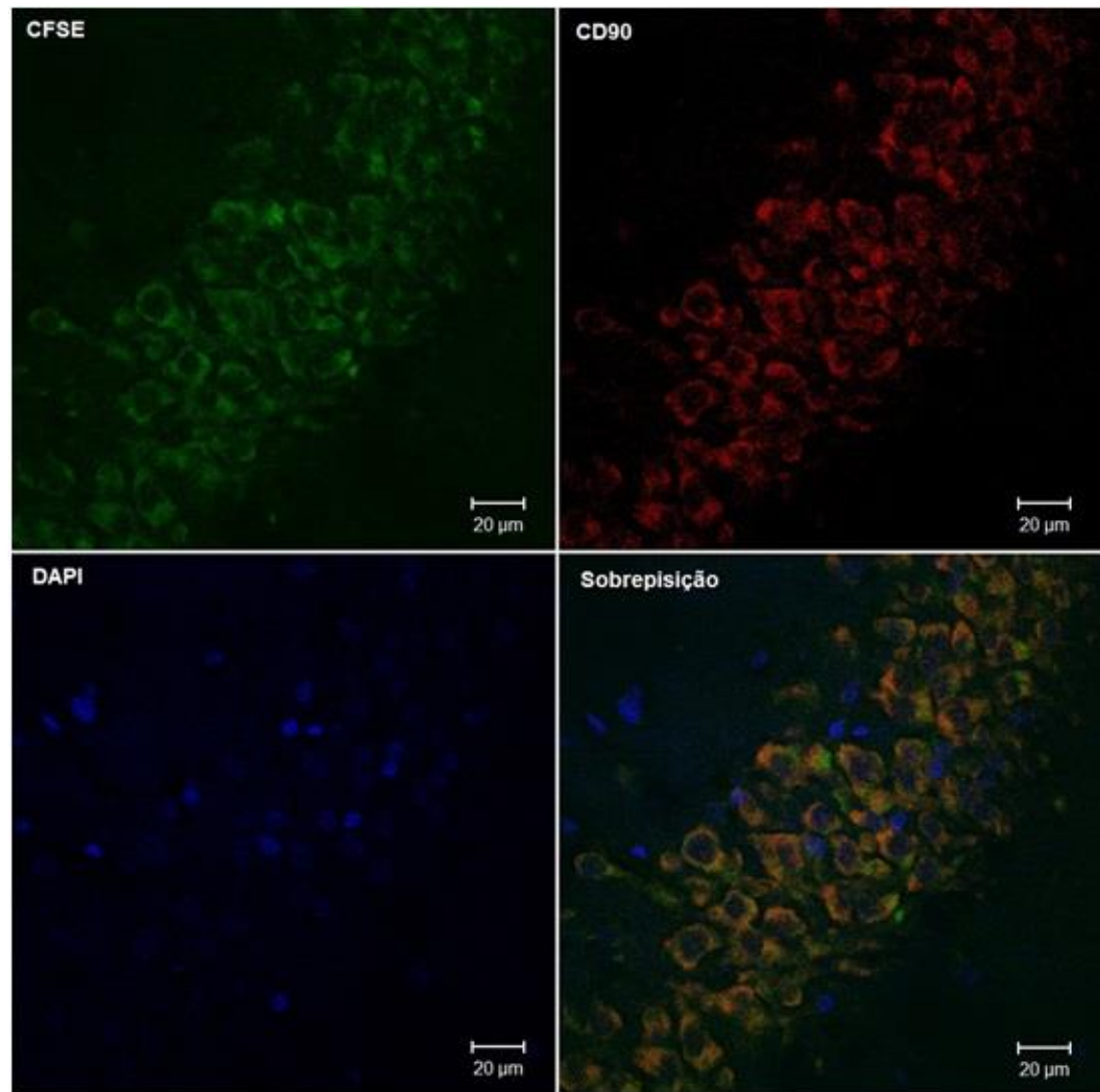
Redox regeneration to normal levels

Reduction of apoptosis: bax/bcl-2 ~ normal after MSCs therapy



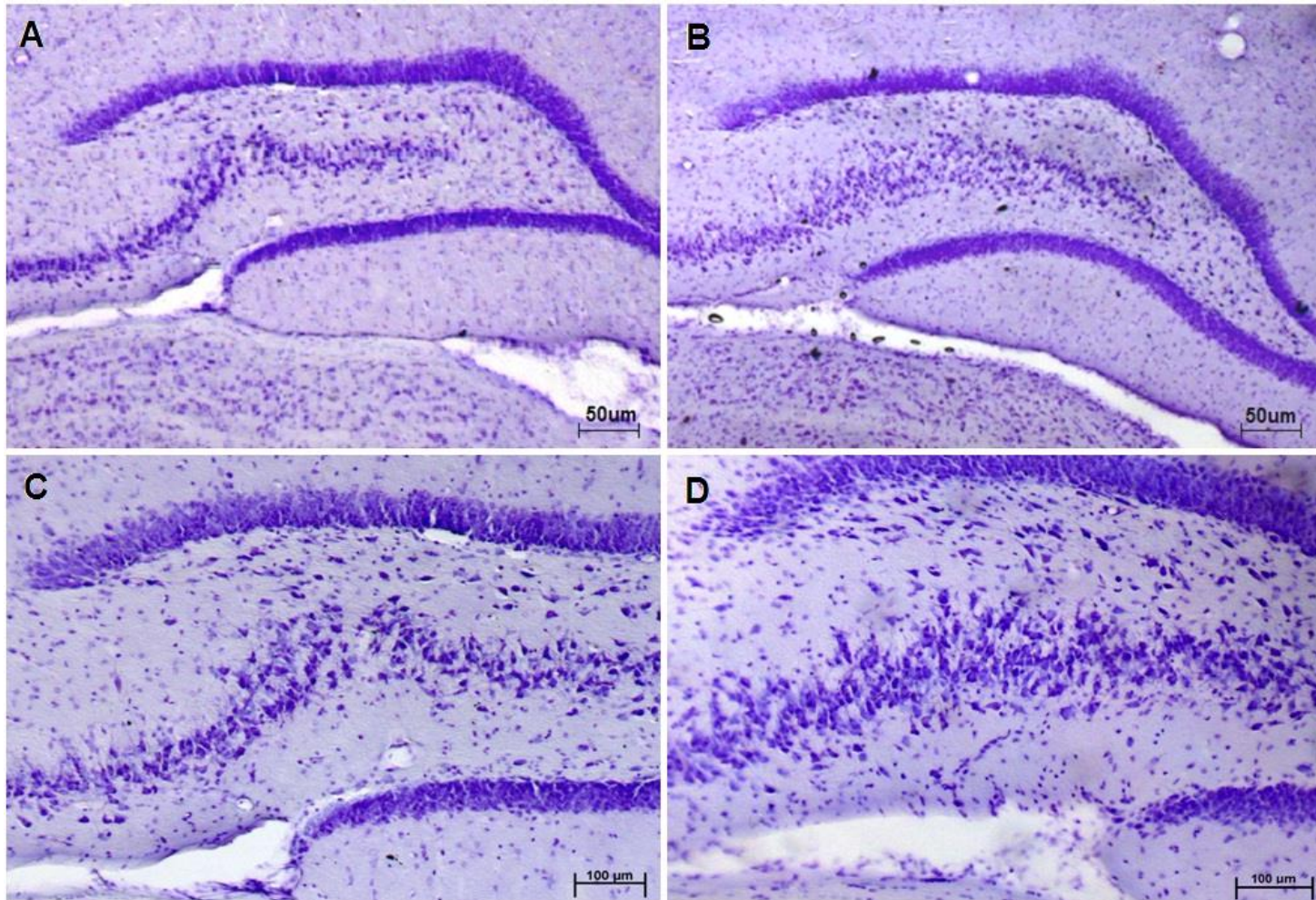
Analysis of gene expression of bax and bcl-2 in brain tissue of WKY and SHRSP in different ages, before and after MSC treatment. **(A)** Representative ethidium bromide electrophoresis gel of RT-PCR; **(B)** qRT-PCR gene expression of bax. **(C)** qRT-PCR gene expression of bcl-2. Note that bax expression increases with age, whereas bcl-2 decreases, in hypertensive rats but not in normotensive rats. Despite The MSC does not decrease the expression of bax, bcl-2 expression was increased in treated animals; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

**Expression of CD90,
a marker of
undifferentiated
cells, in SHRSP rat
brain one month
after MSC
transplantation**



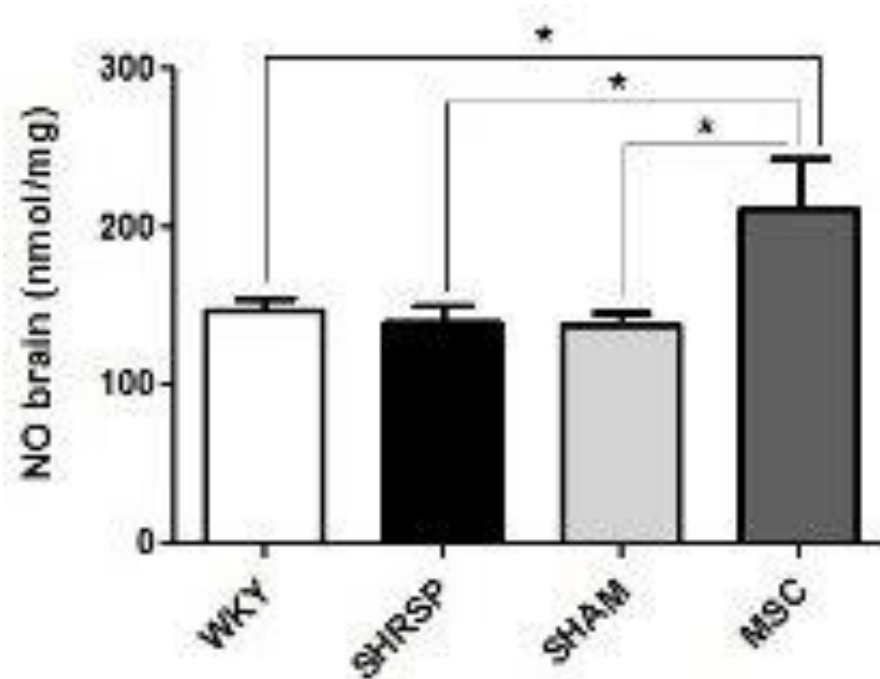
Photomicrograph of animal brains treated with MSCs. Mesenchymal cells labeled with CFSE (green) and injected into the cisterna magna did not differentiate into neurons, at least thirty days after injection and showed labeling for CD90 (red). The nuclei are stained blue with DAPI.

Regeneration of hippocampal injury site in brain of Stroke prone animals after stem cells transplantation



Photomicrographs of coronal sections of rat brains at the level of the hippocampus; dentate gyrus stained by Nissl technique (cresyl). We note that there is no damage and disarray of cell layers in the in treated animals. A and B, 40x magnification. C and D, 100x magnification.

Is Nitric oxide involved in brain damage regeneration?

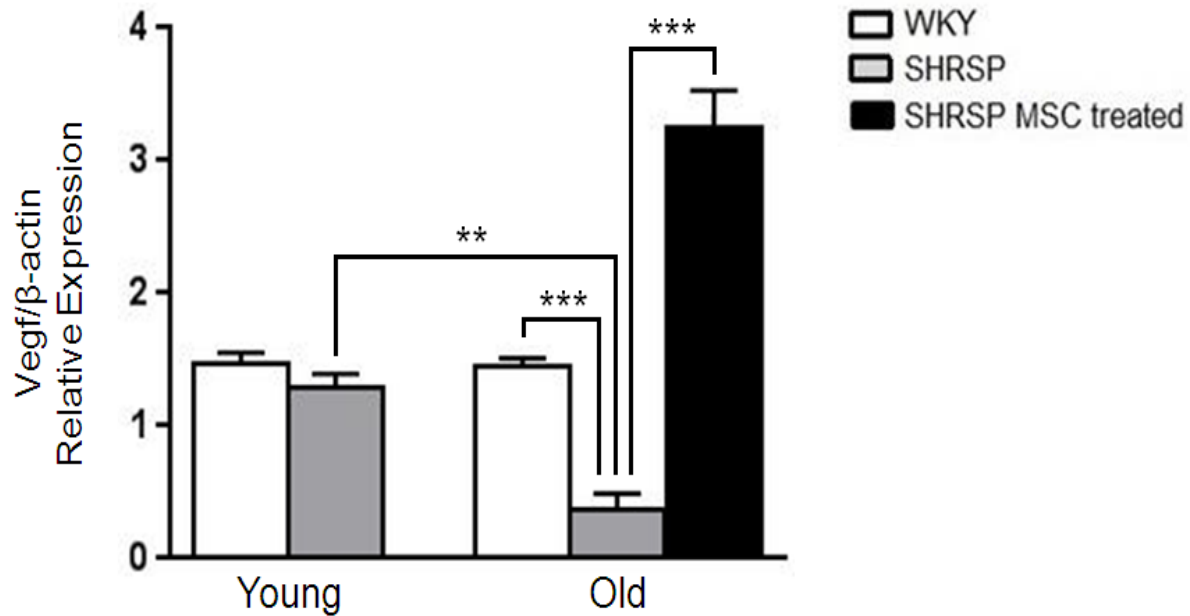


Significant elevation of nitric oxide (NO) in brain of stroke prone rats, 30 days after Stem cells transplantation. n = 10 / group. Values are means \pm SE. *p < 0.01



MSCs from healthy animals can secrete and / or donate nitric oxide to inactivate superoxide in endogenous brain cells of stroke model.

Is the proliferative factor Vegf involved in brain damage regeneration?



Conclusions:

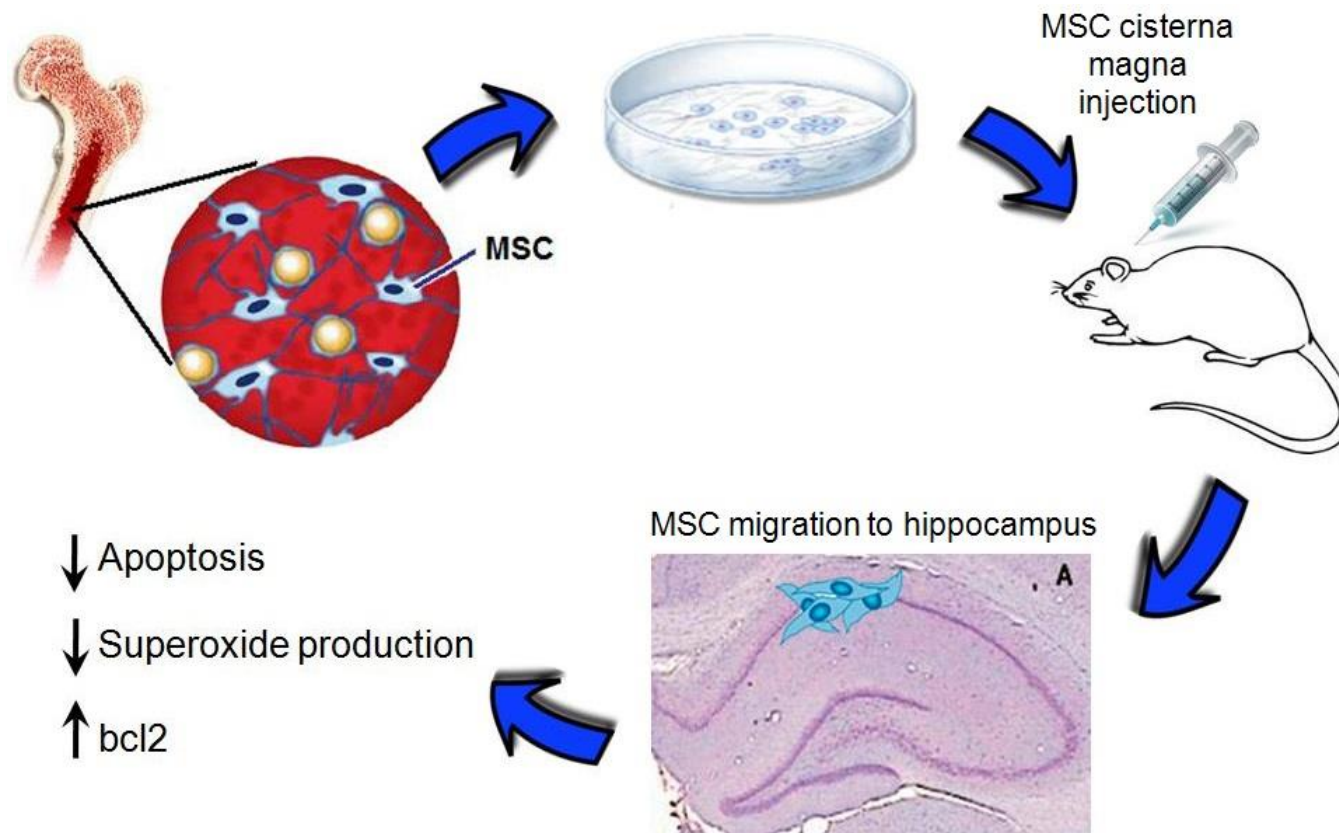
- Mesenchymal stem cells (MSCs) from stroke prone and hypertensive animals have lower viability when compared with normotensive animals;
- When injected into the brains of hypertensive animal model of stroke, MSCs decreased neuronal death by reducing apoptotic rate of these cells, mainly due to the reestablishment of Bcl-2;
- MSCs reduced oxidative stress to **healthy levels in brain** of SHRSP, which indicates a potential antioxidant activity of these cells;
- MSCs were able to reestablish the rearrangement of cell layers in the hippocampus region of stroke prone animals;
- Nitric oxide and Vegf - secreted or induced by transplanted MSCs - are factors involved in brain damage regeneration.



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Transplantation of bone marrow mesenchymal stem cells decreases oxidative stress, apoptosis, and hippocampal damage in brain of a spontaneous stroke model.

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