SUPPLEMENTARY MATERIAL

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Protein extraction

Whole brains were immediately frozen at -80°C after being harvested. Brains were lyzed in ice cold RIPA buffer (50 mM Tris-HCl, 150 mM NaCl, 0.1% sodium dodecyl sulfate (SDS), 1% Triton X-100, 1 mM EDTA, pH 7.4) containing protease inhibitors (Complete mini, Roche Diagnostics France, Meylan, France). Lysates were incubated for 2 hours at 4°C with stirring. Then, lysates were sonicated for 1 minute and kept on ice for another minute between each pulse; this procedure was repeated 3 times. Then, lysates were centrifuged at 10 000 g during 10 minutes and supernatants were collected. Protein concentrations were measured with the Bicinchoninic Acid Kit for Protein Determination (BCA1, Sigma Aldrich, France).

Western blot analysis

Thirty µg of total protein from lysates were loaded on 4-12% sodium dodecyl sulfate–polyacrylamide electrophoretic gel, and transferred onto a polyvinylidenedifluoride membrane. Nonspecific binding was blocked by immersing the membrane in 5% non-fat dry milk in TBST (20 mM Tris HCl, 150 mM NaCl, pH 7.4) for 2 hour, with gentle shaking. After saturation, the membrane was incubated overnight at 4°C with a primary antibody directed against occludin (1:1000 dilution; 33-1500, Fisher Scientific, Illkirch, France), claudin-5 (1:750 dilution; SAB4502981, Sigma-Aldrich, Saint Quentin-Fallavier, France) or zonula occludens-1 (1:750 dilution; 61-7300, Fisher Scientific, Illkirch, France) and actin (loading control, 1:1000 dilution; D6A8, Cell Signalling, Yvelines, France). After washing with TBST, membrane was further incubated with the secondary peroxidase-conjugated antibody (Beckman Coulter, Marseille, France) at a 1:2000 dilution for 1 hour at room temperature. Membranes were washed with TBS and revealed by chemoluminescence (ECL Western blotting substrate, Pierce, Courtaboeuf, France) and the gel image was captured using the Syngene GBox (Ozyme, Saint Quentin en Yvelines, France). Densitometry analyses of chimiluminescence staining were performed with the software *geneSys* (Ozyme, Saint Quentin en Yvelines, France) and results were normalized with values obtained with actin.

Neurobehavioral test

The modified neurological severity score (mNSS) was used to evaluate the neurological function of animals through several previously described tests[1]. This score ranges from 0 to 18, with 0 representing the absence of neurological deficit and 18 being the maximum deficit. This test was carried out on D29. The foot-fault test was used to evaluate the sensitivomotor function of animals. The rats were invited to walk on a horizontal scale of 77 bars. The bars were spaced two centimetres (cm) apart. A computer-assisted system (Locotronic, Bioseb®) records the wrong steps (passage of a paw between two bars). The animals were habituated to walk the ladder and three rungs of the ladder were removed during the

evaluation. We measured the number of faults and the time required to cross the entire corridor. The animals performed this test on D29.

We used the adhesive removal test[2] to determine the effects of brain damage on sensory-motor behaviour. The adhesive paper was placed so that it surrounds the paw of the animal which is subsequently placed in a cage. We measured the latency to realize the presence of the adhesive paper, and the time taken to remove it (in seconds). Each animal was tested four times, twice with the adhesive placed on each foreleg. After each pass, the cage was cleaned. The test ended when the animal removes the adhesive from its paw or the three minutes have elapsed. The somatosensory deficits were graded on a score of 0 to 5 (as described by Moubarik et al.[3] with slight modifications): [0: the animals do not feel the attached adhesive and do not remove it; 1: the animals feel the adhesive but cannot remove it within 3 minutes; 2: the animals remove the adhesive between 2 and 3 minutes; 3: the animals removing the adhesive between 1 and 2 minutes; 4: the animals removing the adhesive between 10 and 60 seconds; 5: animals removing the adhesive between 0 and 10 seconds]. This test was carried out at D29.

Supplementary results

Impact of adenine rich diet on weight, food consumption and blood pressure in rats

The weight curves of the rats are presented in **Supplementary Figure 1A**. Average weekly food consumption by rat was 168.1±12.1 g in the control group *vs.* 149.3±10.1 g in the 0.25% ARD group (*P*=0.20) and 92.0±16.7 g in the 0.5% ARD group (n=8; *P*<0.0001). At D28, both systolic and diastolic blood pressures were higher in the 0.5% ARD group than in the control group: 157.3±11.6 mmHg and 130.0±9.9 mmHg respectively (n=6; *P*=0.004) and 97.8±13.6 mmHg and 80.5 mmHg±10.2 mmHg (n=6; *P*=0.026), respectively.

Adenine rich diet induced dose dependent biological CKD phenotypes

There was a significant increase in plasma creatinine over time compared with the control group in the 0.5%ARD group (n=8; *P*=0.0004) but not in the 0.25%ARD group (n=8; *P*=0.64). At day 28, the average creatinine was 39.5±3.0 µmol/L in the control group *vs.* 55.5±5.4 µmol/L in the 0.25%ARD group and 157.8±31.9 µmol/L in the 0.5%ARD group (*P*=0.048 and *P*<0.0001, respectively; n=8). There was a significant increase in plasma urea over time compared to the control group in the 0.5%ARD group (*P*=0.0002) but not in the 0.25%ARD group (n+8; *P*=0.81). At day 28, the average urea was 6.7±1.0 mmol/L in the control group *vs.* 13.7±1.9 mmol/L in the 0.25%ARD group (*P*=0.043) and 42.1±8.8 mmol/L in the 0.5%ARD group (*P*<0.0001); n=8 (Supplementary Figure 1B, 1C).

Renal failure did not induced sensorimotor nor somato-sensorial impairment

The mNSS score was not different between the control and 0.5%ARD groups (Supplementary Figure 2A). The number of faults and the crossing time at the foot-fault test were not different between the control and 0.5%ARD groups (Supplementary Figures 2B, 2C). The ART score was not different between the control and 0.5%ARD groups for the right and left paws (Supplementary Figure 2D).

0.5%ARD did not modify the expression of tight junction proteins, occludine, claudin5 and ZO-1 in rat brains compared to control (Supplementary Figure 3).

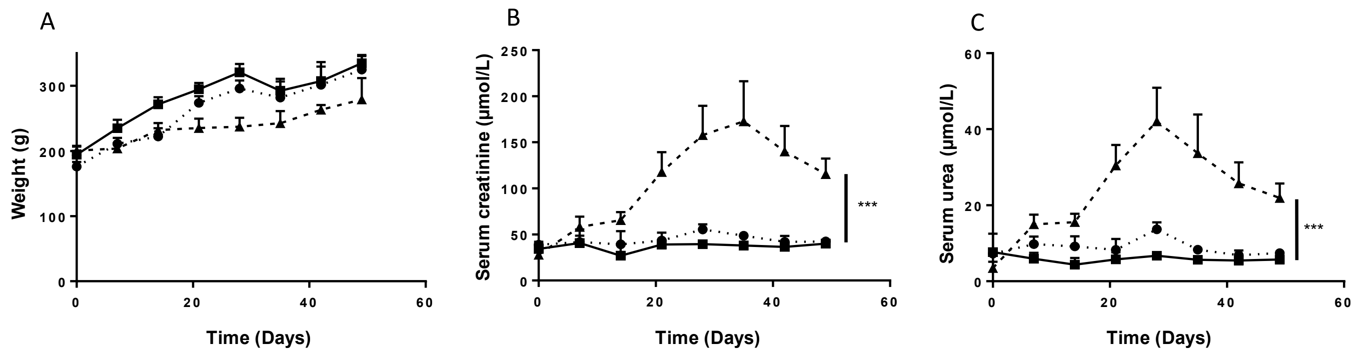
Supplementary references

1. Chen J, Li Y, Wang L, Zhang Z, Lu D, Lu M, Chopp M: Therapeutic benefit of intravenous administration of bone marrow stromal cells after cerebral ischemia in rats. *Stroke* 32: 1005–1011, 2001

2. Schallert T, Upchurch M, Wilcox RE, Vaughn DM: Posture-independent sensorimotor analysis of inter-hemispheric receptor asymmetries in neostriatum. *Pharmacol. Biochem. Behav.* 18: 753–759, 1983

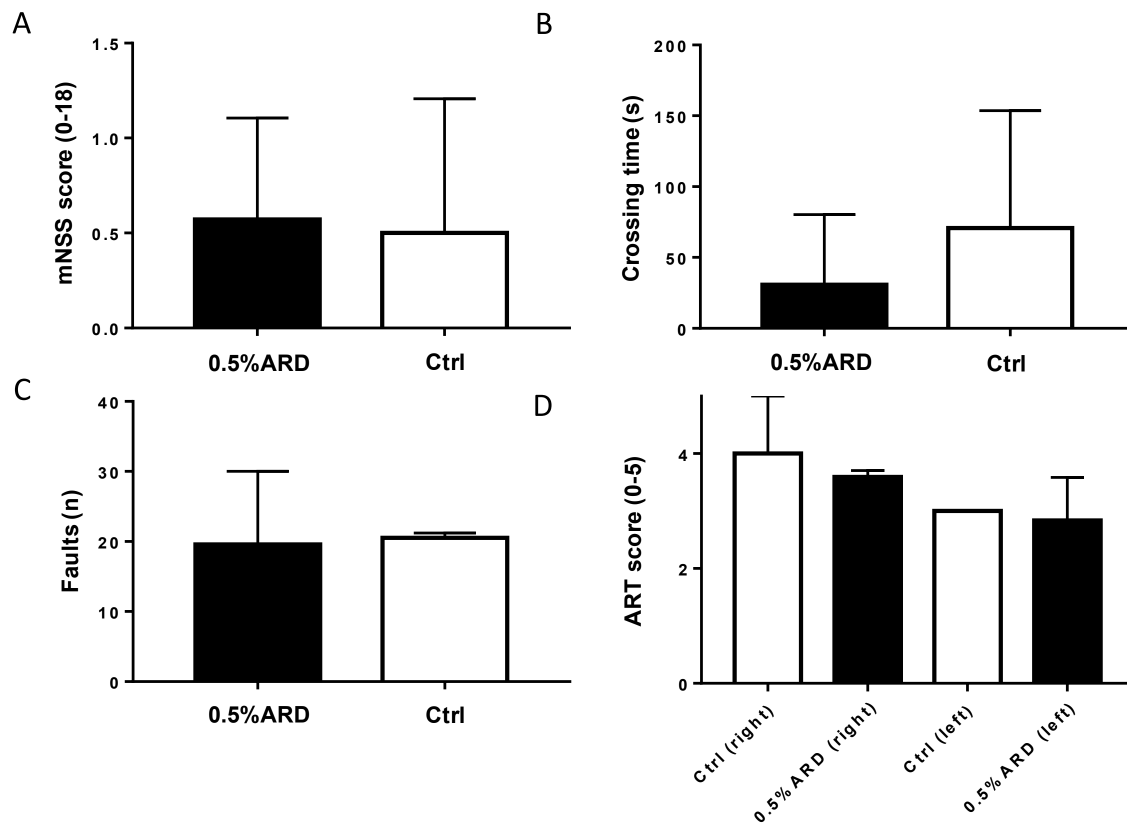
3. Moubarik C, Guillet B, Youssef B, Codaccioni J-L, Piercecchi M-D, Sabatier F, Lionel P, Dou L, Foucault-Bertaud A, Velly L, Dignat-George F, Pisano P: Transplanted late outgrowth endothelial progenitor cells as cell therapy product for stroke. *Stem Cell Rev.* 7: 208–220, 2011

Supplementary Figure 1



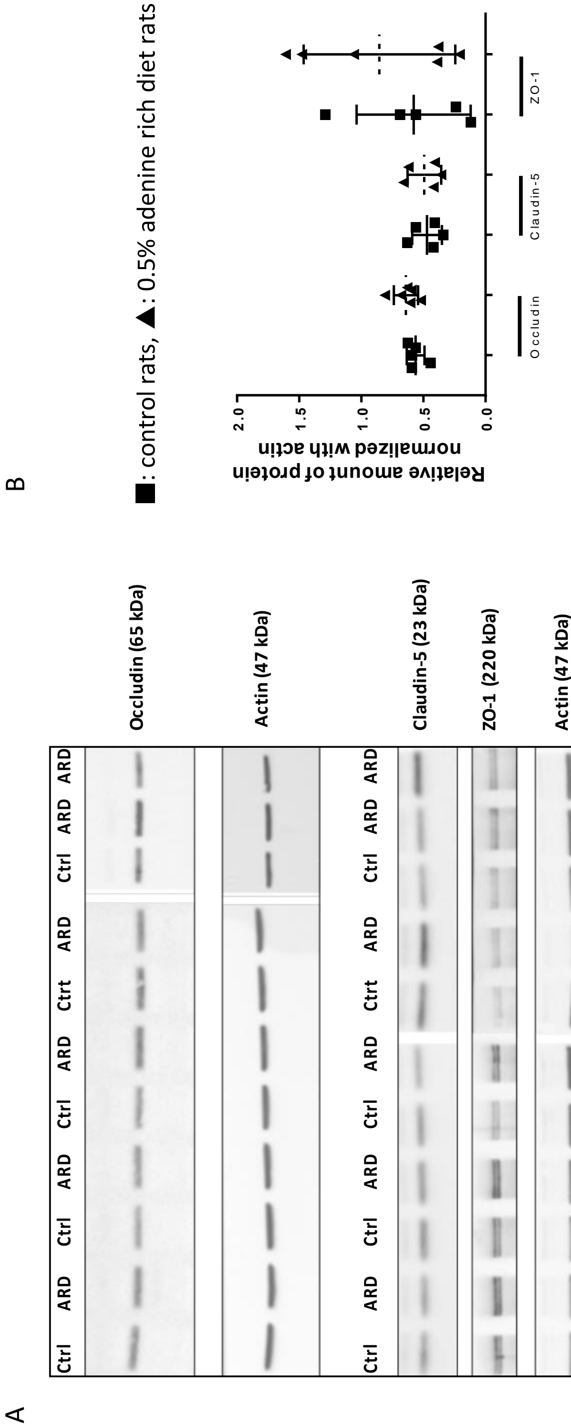
Suppl Figure 1: Clinical and biological evaluation of control or 0.25% and 0.5% Adenine Rich Diet rats. (A) Weight variation over time, (B) Serum creatinine over time, (C) Serum urea over time, ***: $P<0.001$

Supplementary Figure 2



Suppl figure 2: Neurological evaluation of rats fed with control (Ctrl) or 0.5% Adenine Rich Diet (ARD) (A) mNSS score, (B) Crossing time during Foot-Fault Test, (C) Number of faults during Foot-Fault Test, (D) Adhesive Removal Test (ART) score

Supplementary Figure 3



Suppl figure 3: Western blot analysis of occludin, claudin-5 and zonula occludens-1 (ZO-1) expressions in brains of control or 0.5% adenine rich diet (ARD) rats. (A) representative blots, (B) relative quantification.