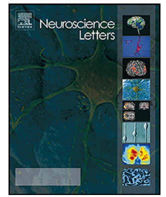
Neuroscience Letters 631 (2016) 50–55

Contents lists available at ScienceDirect

Neuroscience Letters

journal homepage: www.elsevier.com/locate/neulet



Research article

Human endothelial progenitor cells rescue cortical neurons from oxygen-glucose deprivation induced death

Susanna Bacigaluppi (MD, PhD)[a,b,*], Elisabetta Donzelli (PhD)[a,c], Valentina De Cristofaro[a], Nicola Luigi Bragazzi (MD, PhD)[d], Giovanna D'Amico[e], Arianna Scuteri (PhD)[a,c], Giovanni Tredici (MD)[a]

[a] School of Medicine and Surgery, University of Milano-Bicocca, Monza, Italy
[b] Department of Neurosurgery, Galliera Hospital, Genova, Italy
[c] Milan Center for Neuroscience (NeuroMI), Milan, Italy
[d] School of Public Health, Department of Health Sciences (DISSAL), University of Genova, DINOGMI, Genova, Italy
[e] Centro Ricerca Tettamanti, Department of Pediatrics, University of Milano-Bicocca, Fondazione MBBM/San Gerardo Hospital, Monza, Italy

HIGHLIGHTS

- Neuronal ischemia was studied in vitro with OGD on a primary rat embryonic cortical neuron culture.
- At day 2, after 2 hrs OGD, neuronal survival without treatment was impaired.
- At day 2, after 2 hrs OGD, treatment with indirect co-cultures with hEPC or hMSC significantly increased neuronal survival.
- Conditioned medium by hEPC or hMSC did not provide any advantage in terms of survival to OGD neurons.

ARTICLE INFO

Article history:
Received 6 April 2016
Received in revised form 12 July 2016
Accepted 9 August 2016
Available online 10 August 2016

Keywords:
Oxygen glucose deprivation
Cortical neurons
Endothelial progenitor cells
Mesenchymal stem cells
Indirect co-culture

ABSTRACT

Background and aim: Cerebral ischemia is characterized by both acute and delayed neuronal injuries. Neuro-protection is a major issue that should be properly addressed from a pharmacological point of view, and cell-based treatment approaches are of interest due to their potential pleiotropic effects. Endothelial progenitor cells have the advantage of being mobilized from the bone marrow into the circulation, but have been less studied than other stem cells, such as mesenchymal stem cells. Therefore, the comparison between human endothelial progenitor cells (hEPC) and human mesenchymal progenitor cells (hMSC) in terms of efficacy in rescuing neurons from cell death after transitory ischemia is the aim of the current study, in the effort to address further directions.
Materials and methods: In vitro model of oxygen-glucose deprivation (OGD) on a primary culture of rodent cortical neurons was set up with different durations of exposure: 1, 2 and 3 hrs with assessment of neuron survival. The 2 hrs OGD was chosen for the subsequent experiments. After 2 hrs OGD neurons were either placed in indirect co-culture with hMSC or hEPC or cultured in hMSC or hEPC conditioned medium and cell viability was evaluated by MTT assay.
Results: At day 2 after 2 hrs OGD exposure, mean neuronal survival was 47.9 ± 24.2%. In contrast, after treatment with hEPC and hMSC indirect co-culture was 74.1 ± 27.3%; and 69.4 ± 18.8%, respectively. In contrast, treatment with conditioned medium did not provide any advantage in terms of survival to OGD neurons
Conclusion: The study shows the efficacy of hEPC in indirect co-culture to rescue neurons from cell death after OGD, comparable to that of hMSC. hEPC deserve further studies given their potential interest for ischemia.

© 2016 Elsevier Ireland Ltd. All rights reserved.

Abbreviations: hEPC, human endothelial progenitor cells; hMSC, human mesenchymal stem cells; OGD, oxygen-glucose deprivation; MTT assay, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay.

* Corresponding author at: Department of Neurosurgery, E.O. Ospedali Galliera, Mura delle Cappuccine 14,10168 Genova, Italy.
E-mail address: susannabacigaluppi@yahoo.it (S. Bacigaluppi).

http://dx.doi.org/10.1016/j.neulet.2016.08.014
0304-3940/© 2016 Elsevier Ireland Ltd. All rights reserved.

1. Introduction

Ischemia can have several origins, including embolism [51], traumatic brain injury [47], cardiac arrest [54], vasospasm [5,37], various hemodynamic causes [31], and temporary vessel occlusion during cerebro-vascular surgery [4], among others. Despite this heterogeneity of etiological causes, the commonly shared feature of brain ischemic injury is the resulting neuronal loss as a consequence of oxygen and glucose deprivation (OGD) [22].

Ischemic brain damage is characterized by both acute and delayed neuronal injuries. Delayed neuronal death is a relatively slow process, taking 2–3 days to occur [29]. The time window of delayed neuronal death offers a potential opportunity for the application of some measures aimed at improving or even reversing the latter phenomenon [28].

While mechanisms of early ischemic response are in great part well explained by the glutamate-Ca^{2+} theory, delayed phenomena involve several pathways [13,22,28].

Thus, an ideal treatment should target multiple mechanisms in order to be effective. Beyond the issue regarding the time-window for reperfusion of ischemic brain aimed at reducing the stroke penumbra, whose definition criteria are continuously improved and redefined [12], there are several pharmacological approaches aimed at providing neuro-protection [50].

Stem cells have been recognized as therapeutic tools due to their multiple positive effects in stroke, including their ability of homing to the infarcted area, and their neuro-regenerative capacities, vasculogenic properties and modulating effects on the inflammatory reaction [9]. In the field of ischemia, most studies are in vivo and mainly focus on the effects of stem cells and their products towards the inflammatory and angiogenic response. Furthermore, most of the studies are based on either neuronal stem cells or mesenchymal stem cells. Both of these require exogenous administration [42]. In the last decade endothelial progenitor cells (EPC) have attracted some attention in the cardiovascular field first [16] and, more recently, also in the field of cerebrovascular disease [49], among others. They appear of great interest both for presumable bystander effects on injured parenchyma and for direct effects on the micro-vascular system, though these discoveries are still in progress [36]. An interesting aspect is that, differently from other stem or progenitor-like cells, EPC can be mobilized from the bone marrow in the blood stream by means of endogenous factors such as VEGF [3] as well as drugs such as statins [52] and proved to have a homing at sites of injuries [53]. The present study is aimed at testing in vitro the effect of human endothelial progenitor cells (hEPC) in protecting cultured cortical neurons from cell death and at comparing the efficacy of hEPC to that of human mesenchymal stem cells (hMSC).

2. Materials and methods

2.1. Rat embryonic cortical neurons

Primary cultures were established from E18 Sprague Dawley rats (Harlan, Italy) accordingly to a previously published protocol [38]. Briefly, cortices were dissected and digested with 0.25% trypsin and 1% DNase. Cortical neurons were recovered by centrifugation and plated $1.5 \times 10^5/cm^2$ on poly-lysin (0.1 mg/mL, Sigma-Aldrich Co., St. Louis, Missouri, USA) coated dishes in Neurobasal Medium A (Life Technologies, Italy), supplemented with 5% Horse Serum (Euroclone, Italy) and NeuroMix (Euroclone). Neuronal cultures were purified by 24 hrs 2 μM cytosine arabinoside (AraC) treatment. All animal procedures were conducted in conformity with the institutional guidelines, in compliance with both national (DL 26/2014) and international (EEC Council Directive 2010/63) laws and policies. The protocol was approved by the Ethics Committee of the University Milano-Bicocca (protocol n. 0035828/13).

2.2. hMSC

hMSC (passages 7–9) were obtained from the "Stefano Verri" Cell Therapy Laboratory, Monza, Italy. Briefly, cells were isolated from aliquots of heparinized bone marrow obtained in excess from three healthy individuals undergoing marrow harvest for allogenic transplantation at the San Gerardo Hospital (Monza, Italy) after patients' understanding and informed consent. They were cultured in Dulbecco's Modified Eagle Medium (DMEM) (Euroclone) supplemented with 10% Fetal Bovine Serum (Hyclone, USA) [14].

2.3. hEPC

hEPC (ABCell-Bio, France) were cultured in EGM-2 medium (Lonza, Switzerland) and used at passages 6–10.

2.4. OGD

Neurons were incubated in DMEM, without glucose, in a hypoxia incubator chamber (Billups - Rothemberger, USA) in 95% N_2-5% CO_2 for 1, 2 and 3 hrs. At the end of OGD treatment, neurons were returned to normoxia and medium was replaced with neuronal medium [27].

2.5. Cell counting

Pictures of the same field of neurons plated on gridded dishes were taken at day 0 and at day 2 after OGD experiments with Leica DFC290 camera connected to an optical microscope. The viable neurons, visible as birifrangent cells, were manually counted and the percentage of neurons with respect to the initial neuronal number (day 0) was calculated and normalized with the counts of the control neurons at the same time-points. Experiments with cell counting were repeated two times.

2.6. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay

To assess viability the MTT assay, which quantifies mitochondrial activity [40], was performed at day 2 after OGD. Cultures were incubated at 37 °C for 2 hrs with 0.5 mg/mL MTT (Sigma Aldrich). Formazan crystals were solubilized in ethanol and absorbance was measured at 560 nm with 690 nm background subtraction. Experiments with MTT-assay were repeated four times for the set up of different timings and 5 times to obtain at least four experimental replicates for each experimental point.

Results are provided at each time-point as percentage with respect to control neurons.

2.7. Indirect co-cultures

5×10^4 hMSC or hEPC were plated on 12-well polyester inserts with 3 μm pore size (Corning). After 24 hrs the standard culture medium was replaced with the neuronal medium. The day after, the neurons were treated with OGD for 2 hrs and subsequently the hMSC and the hEPC plated on the inserts were transferred on the neurons and the medium was replaced with fresh neuronal medium. Neuronal survival was assessed by MTT-assay on day 2 after OGD.

Download English Version:

https://daneshyari.com/en/article/6279099

Download Persian Version:

https://daneshyari.com/article/6279099

Daneshyari.com