Evaluation of the Safety and Efficacy of the Therapeutic Potential of Adipose-Derived Stem Cells Injected in the Cerebral Ischemic Penumbra

Ulises Gómez-Pinedo, PhD,[*,1] Leyre Sanchez-Rojas, DMV,[*,1] María Soledad Benito-Martin, PhD,[*] Cristina Lendinez, MDV,[*] Gonzalo León-Espinosa, PhD,[*] Fernando Rascón, MD,[†] Jone Herrero, PhD,[‡] Begoña Castro, PhD,[‡] Lidia Moreno-Jiménez, PhD,[*] Maite del Olmo, PhD,[‡] Jordi A. Matias-Guiu, MD, PhD,[§] Jorge Matias-Guiu, MD, PhD,[§] and Juan A. Barcia, MD, PhD[†]

Introduction: Stroke represents an attractive target for cell therapy. Although different types of cells have been employed in animal models with variable results, the human adipose-derived stem cells (hASCs) have demonstrated favorable characteristics in the treatment of diseases with inflammatory substrate, but experience in their intracerebral administration is lacking. The purpose of this study is to evaluate the effect and safety of the intracerebral application of hASCs in a stroke model. *Methods:* A first group of Athymic Nude mice after stroke received a stereotactic injection of hASCs at a concentration of $4 \times 10^4/\mu L$ at the penumbra area, a second group without stroke received the same cell concentration, and a third group had only stroke and no cells. After 7, 15, and 30 days, the animals underwent fluorodeoxyglucose-positron emission tomography and magnetic resonance imaging; subsequently, they were sacrificed for histological evaluation (HuNu, GFAP, IBA-1, Ki67, DCX) of the penumbra area and ipsilateral subventricular zone (iSVZ). *Results:* The in vitro studies found no alterations in the molecular karyotype, clonogenic capacity, and expression of 62 kDa transcription factor and telomerase. Animals implanted with cells showed no adverse events. The implanted cells showed no evidence of proliferation or differentiation. However, there was an increase of capillaries, less astrocytes and microglia, and increased bromodeoxyuridine and

From the *Laboratorio de medicina regenerativa, Instituto de Neurociencias, Instituto de Investigación Sanitaria San Carlos (IdISSC), Hospital Clínico San Carlos, Madrid, Spain; †Servicio de Neurocirugía, Instituto de Investigación Sanitaria San Carlos (IdISSC), Hospital Clínico San Carlos, Universidad Complutense de Madrid, Madrid, Spain; ‡Histocell S.L., San Sebastián, Spain; and §Servicio de Neurología, Instituto de Investigación Sanitaria San Carlos (IdISSC), Hospital Clínico San Carlos, Universidad Complutense de Madrid, Madrid, Spain.

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Address correspondence to Juan A. Barcia, MD, PhD, Servicio de Neurocirugía, Hospital Clinico San Carlos, Calle Prof. Martin Lagos s/n, Madrid 28040, Spain. E-mail: jabarcia@ucm.es.

[1] Both authors contributed equally to this work.

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doublecortin-positive cells in the iSVZ and in the vicinity of ischemic injury. *Conclusions:* These results suggest that hASCs in the implanted dose modulate inflammation, promote endogenous neurogenesis, and do not proliferate or migrate in the brain. These data confirm the safety of cell therapy with hASCs. **Key Words:** Stroke—ASC therapy—brain surgery—stem cells—animal model—intracerebral transplantation.

Introduction

Brain stroke is one of the most important conditions in health care, not only because of its high incidence, making it 1 of the 3 more frequent causes of mortality and the first one of disability in the western world, but also because of the high cost to the health-care system due to treatment, rehabilitation, and prevention of further episodes. Even if stroke treatment in the acute phase has improved ostensibly during the last decade, it is still a clinical situation provoking important neurological sequelae, which can lead to physical disability and dementia. Because of this, the search for alternative treatments devoted to the recovery of patients with stroke is a priority objective in clinical research.

The finding after experimental stroke of increased neurogenic activity, presence of endogenous neural stem cells at the cortex, and proliferation of nestin-positive cells both at the periphery and at the core of the ischemic zone[1,2] and of the survival of these cells at the ischemic area after permanent ischemia,[3-5] has boosted the idea that cell therapy may be effective for these patients. Different experimental studies support this possibility. The implant of stem cells induces a functional improvement in experimental stroke[6-10] and after the implant of immortalized pluripotent teratoma cells.[11] There are many possible mechanisms that could explain the reduction in infarct volume and neurological deficits found after ischemic stroke, such as replacement of neurons, neuroprotection, cell rescue via trophic support, promotion of endogenous neurogenesis, immunomodulation, or axonal plasticity, although the most probable one seems to be a local neuroprotective effect.[12] However, different experimental studies have shown that the generation of angiogenesis in the area close to the ischemia parallels the functional improvement in experimental stroke. The use of stem cells of mesenchymal origin has demonstrated to induce angiogenesis, and it could stimulate neurogenesis and the migration of neural cells to the ischemic area.[13]

It is because of this that the use of cells of mesenchymal origin has been proposed as a therapeutic possibility in stroke.[14] Among the mesenchymal stem cells, those derived from adipose tissue present a series of both biological and practical advantages making them interesting candidates for their therapeutic use. One of these advantages is that they induce angiogenesis through the liberation of cytokines as the vascular endothelial growth factor (VEGF).[15] Moreover, they present pathotropism especially to hypoxic areas. However, immunomodulatory effects have been described for mesenchymal cells both in vitro (they do not induce allogenic lymphocytic response and inhibit the lymphocytic response provoked by mitogens)[16,17] and in vivo (mesenchymal cells have controlled the graft versus host reaction associated to hematopoietic transplantations).[18]

Furthermore, these cells are widely available for their allogenic use, given the number of lipoaspirates done nowadays in plastic surgery, facilitating its donation. Different companies may provide cells from healthy donors, adequately processed and packed for their immediate use in humans. These characteristics make adipose tissue-derived stromal cells (ASCs) perfect candidates for stem cell therapy in stroke, and these cells have been used in various studies in experimental stroke.[19-29]

Some of the problems for the use of transplanted cells in the clinical treatment of stroke are the access pathway for the cells to the injured area and the understanding of the mechanisms of action. Although several studies have tested the use of cells delivered by the intravenous, intra-arterial, or intrathecal routes,[30-32] and the transplanted cells can migrate to the lesion area, their access to the area is difficult and their location in the penumbra zone is very sparse, which may foreshadow a beneficial effect. However, little is known about the mechanisms these cells could use to improve the conditions of the lesioned area when transplanted into the ischemic penumbra area. In addition, it is important to elucidate the safety aspects of this delivery, in terms of neurological, teratogenic, and inflammatory side effects.

In consequence, the objective of this study is to demonstrate the efficacy and safety of the direct brain injection of allogenic ASC in a stroke model administered into the ischemic penumbra area to proceed to translational clinical research.

Materials and Methods

Adipose Tissue Collection

Adipose tissue samples were obtained from donors during routine abdominoplasty following informed patient consent and according to the guidelines set by the corresponding ethics committee on biomedical research. The adipose tissue was transported in sterile bottles and was immediately