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Review

Clinical application of adult olfactory bulb ensheathing glia for nervous system repair

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ABSTRACT

The ability of adult olfactory bulb ensheathing glia (OB-OEG) to promote histological and functional neural repair has been broadly documented. Pre-clinical studies show that beneficial effects of adult OB-OEG are repeatable in the same type of spinal cord injury initially tested, in other spinal cord and CNS injury models, in different species and after the administration of these cells in different forms (either alone or in combination with other cells, drugs, products or devices). These studies demonstrate the reproducibility, robustness, fundamental nature and relevance of the findings. Therefore, the use of adult OB-OEG for spinal cord injury repair meets the scientific criteria established by the International Campaign for Cures of Spinal Cord Injury Paralysis (ICCP) for the translation to human application.
Because there is so much heterogeneity in the way adult OEG is administered, each of these different OEG-based therapies must be individually categorized to determine whether they fulfill the requisites dictated by the consolidated regulatory body to be considered or not as a medicine. In the case they do, in Europe, they shall be subjected to the Regulatory European Framework for Advanced Therapy Medicinal Products and the European Clinical Trials Directive (Directives 2001/20/EC and 2009/120/EC). After a deep analysis of the European Regulation we have concluded that grafts consisting of suspensions of purified adult OEG, to be used for the promotion of axonal regeneration in the CNS, do not comply with the definition of Medicinal Product provided by the European Medicines Agency. In contrast, experimental therapies using OEG in combination with other cell types, drugs, products or devices, or genetically-modified OEG fall under the definitions of Medicinal Product. This article is part of a Special Issue entitled: Understanding olfactory ensheathing glia and their prospect for nervous system repair.

Contents

Abbreviations: CATMP, Combined Advanced Therapy Medicinal Product; CBMP, Cell-Based Therapy Medicinal Product; CNS, Central Nervous System; EMP, Engineered Medicinal Product; GTMP, Gene-Therapy Medicinal Product; ICCP, International Campaign for Cures of Spinal Cord Injury Paralysis; SCMP, Somatic Cell Therapy Medicinal Product; OB-OEG, Olfactory bulb ensheathing glia.

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Introduction

Olfactory receptor neurons, located in the epithelium of the nasal cavity, undergo continuous turnover in adult mammals. The newly generated neurons grow their axons across the cribiform plate of the ethmoidal bone, traverse the piamater and enter the Central Nervous System (CNS) of the olfactory bulb (OB). Here they are able to navigate, reach and synapse with the dendrites of mitral, tufted and periglomerular cells in the olfactory glomeruli (Barber, 1982; Doucette et al., 1983; Graziadei and Graziadei, 1979; Graziadei and Monti Graziadei, 1980). Surprisingly, researchers discovered that olfactory axons possessed the ability to elongate and reconnect continuously throughout life, thus making the olfactory bulb a unique CNS structure capable of spontaneous axonal regeneration. The failure of axonal regeneration within the adult mammalian CNS has been attributed to the inhibitory environment created around the axons by the glial cells. As a result, scientists argued that this distinct capacity for axonal regeneration exhibited by the olfactory bulb (OB) and other CNS structures might be related to their unequal cellular composition. Going back to the first histological studies about the OB, it was intriguing to find that in 1875 Golgi (Golgi, 1875) and in 1898 Blanes (Blanes, 1898) described the presence of a fusiform glial type, exclusively located within the olfactory nerve and glomerular layers, which serve to isolate olfactory axons from other CNS cells. This was later confirmed at the ultrastructural level by other scientists (Barber and Lindsay, 1982; Doucette, 1984, 1990; Pixley, 1992; Raisman, 1985; Valverde and Lopez-Mascaraque, 1991; Valverde et al., 1992). They described a Schwann-like glial cell that surrounded and ensheathed olfactory axons through their entire path inside the bulb. This type of macroglial cells was exclusively encountered in a region of the CNS where axonal regeneration was possible during adulthood and, hence, suggested a key role in axonal growth for these cells. Blanes' macroglia was then named *olfactory ensheathing cells* (OEC) or *olfactory ensheathing glia* (OEG) to honor their main property, and because their ability to enfold and isolate olfactory axons from the hostile CNS environment was believed to be linked to their capacity to promote axonal growth.

Adult OEG play a critical role in the continuous regeneration of olfactory axons into the olfactory bulb, prompting scientists to consider their growth promoting properties in other regions of the CNS. If successful a new tool for nervous system repair could be available. To test this hypothesis, first OEG had to be isolated and purified from adult OBs and then these cultures had to demonstrate that their ability to enfold axons remained preserved. Cultures of adult OB-OEG were obtained and characterized (Ramon-Cueto and Nieto-Sampedro, 1992). Both, their *in vivo* and *in vitro* properties suggested that they were a unique glia cell type with axonal growth promoting properties (Ramon-Cueto and Valverde, 1995). Purified adult OB-OEG had to also maintain their ability to promote axonal elongation and ensheathment because these properties were considered essential to exert their positive and potentially therapeutic effect. Co-culture studies confirmed that after isolating OEG from adult bulbs, these cells continued favoring neurite extension and enfoldment. The *in vivo* role of these cells and the demonstration that they retained their properties in culture suggested that adult OB-OEG could be a good tool to foster axonal regeneration within the adult mammalian CNS (Ramon-Cueto and Valverde, 1995).

A region that closely resembles the PNS/CNS olfactory bulb transition zone is the dorsal root entry zone. However, in this region spontaneous axonal regeneration into the spinal cord is not possible (Carlstedt, 1985; Carlstedt et al., 1989; Ramón y Cajal, 1991). Thus, a "proof of principle" study could evaluate whether or not adult OB-OEG favored the entrance of injured root axons across this PNS-CNS interface. Indeed, these axons were able to cross the root-cord boundary, elongate within the CNS environment of the spinal cord and reach the laminae they innervate under normal conditions after OB-OEG grafting (Ramon-Cueto and Nieto-Sampedro, 1994). These positive results encouraged scientists to pursue the next challenge: to determine whether OB-OEG could also exert axonal growth-promoting effect and functional improvement after CNS damage. This would, in turn, determine the real prospect of adult OB-OEG transplants for nervous system repair in humans.

In this article we provide an overview of the legislation currently in force in Europe for cell-based therapies and how this may affect the clinical translation of therapies using OB-OEG grafts for neural repair. We also review the studies that have been carried out so far in animal models using adult OB-OEG as a therapeutic agent for neural repair. Finally we discuss whether or not adult OB-OEG transplants fulfill the guidelines for the translation into clinical application.

Regulatory European framework for cell-based therapies

All new medicinal products have to be validated and approved by an appropriate national regulatory agency. However, not all countries throughout the world have the same ethics and regulations. Consequently, this has resulted in several experimental treatments applied to humans whose safety and efficacy were not validated *in vitro* and in appropriate animal models. An international common regulatory body and/or legislation would safeguard in all nations equal rights for the patients, but this still has to come. The health regulatory agencies in the United States and in the European Community, the Food and Drug Administration (FDA) and the Committee for Medicinal Products for Human Use, respectively, follow similar general guidelines. They intend to protect the public from any risk or harm coming from an unsubstantiated treatment. In the European Community, all Member States have to follow the Directive of the European Parliament and of the Council on the Community code relating to medicinal products for human use (Directive 2001/83/EC). In this Directive, Part IV of Annex I regulates the specific requirements for "advanced therapy medicinal products" (ATMP) which are comprised of gene therapy medicinal products (GTMP), somatic cell therapy medicinal products (SCMP) and tissue-engineered medicinal products (EMP). This Directive was amended by the Regulation EC No 1394/2007 in order to introduce additional provisions that better regulate the use of ATMP in humans. However, during the last years this field has grown exponentially and has been placed at the forefront of innovation, offering new prospects and hope for diseases that currently have no cure. This legislation had to be adapted again to meet the scientific and technical progress of this field. As a result, part IV of Annex I was replaced by the text of Directive 2009/120/EC accordingly (Schneider et al., 2010). This new legislation entered into force in April 2010.

Cell-based medicinal products (CBMP) may include somatic cell therapy (SCMP), gene therapy (GTMP) and also tissue-engineered products (EMP) from either autologous, allogenic or xenogenic sources (Schneider et al., 2010). According to this European pharmaceutical legislation (Directive 2009/120/EC), a cell-based therapy fulfill the criteria of a medicinal product when it "contains or consists of cells or tissues that have been subjected to substantial manipulation so the biological characteristics, physiological functions or structural properties relevant for the intended clinical use have been altered." The following are not considered substantial manipulations: "cutting, grinding, shaping, centrifugation, soaking in antibiotic or antimicrobial solutions, sterilization, irradiation, cell separation, concentration or purification, filtering, lyophilization, freezing, cryopreservation, vitrification" (Annex I of Regulation (EC) No 1394/2007). A cell-based therapy is also a medicinal product when it "consists of cells or tissues that are not intended to be used for the same essential function(s) in the recipient and the donor." When cells have been genetically modified and are intended for regenerating, replacing or repairing human tissue they are included in the category of "tissue-engineered medicinal products" (Schneider et al., 2010). Moreover, they may also be considered "gene therapy medicinal products." Cells (autologous or others) that are subjected to any "substantial manipulation" to achieve the properties relevant for the intended therapeutic use are also