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USE OF EMBRYONIC STEM CELLS TO TREAT SEVERE EYE DISEASES

USO DE CÉLULAS TRONCALES EMBRIONARIAS PARA TRATAR GRAVES ENFERMEDADES OCULARES

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RESUMEN:

Palabras clave:
Células madre embrionarias; células madre pluripotentes inducidas; medicina regenerativa; células de epitelio pigmentario de retina; evaluación ética.

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Antecedentes: El uso de células troncales constituye una de las principales posibilidades terapéuticas en el área de la medicina regenerativa. Recientes ensayos clínicos utilizando células derivadas de troncales humanas, muestran resultados esperanzadores, aunque estos deben ser valorados con la necesaria cautela. Discusión: Algunos medios de comunicación han divulgado resultados de estos ensayos sin la debida prudencia, creando quizá expectativas que no se corresponden con la realidad de los hechos observados. En el presente trabajo se muestran algunos de los recientes avances en el uso de células troncales humanas y especialmente se revisan los realizados en el área oftalmológica, y más concretamente en la enfermedad de Stargardt y la degeneración macular asociada a la edad. Así mismo, se muestran los prometedores estudios con células troncales pluripotenciales inducidas (iPS) dirigidos a obtener epitelio pigmentario de retina y bastones retinianos sensibles a la luz, con resultados preclínicos y clínicos esperanzadores, en las enfermedades oculares anteriormente referidas. Conclusiones: Desde un punto de vista médico, no hay que olvidar que las células del epitelio de la retina trasplantadas pueden causar tumores, ya que se han obtenido a partir de células madre embrionarias, y pueden desencadenar rechazo inmunológico, dado que son heterólogas. Estas consideraciones ponen de manifiesto la incertidumbre ética de los resultados de estos ensayos clínicos, pero, sobre todo, hay que insistir en que cada vez que se utilizan células madre embrionarias, un embrión humano debe ser destruido para obtenerlas, hecho que tiene dificultades éticas objetivas.

ABSTRACT:

Keywords:
Embryonic stem cells, induced pluripotent stem cells,

Background: The use of stem cells in regenerative medicine has major therapeutic potential. Recent clinical trials using cells derived from human stem cells are showing encouraging results, although these should be assessed with the necessary caution. Discussion: Some media have reported the results of these trials without due care, perhaps creating expectations that do not match the reality of the facts. This paper describes some of the recent advances in the use of human stem cells, particularly those made in the area of ophthalmology, and more specifically, in Stargardt's disease and age-related macular degeneration

Keywords:
regenerative medicine, retinal pigmented epithelium cells, ethical assessment.

(AMD). We also present promising studies with induced pluripotent stem cells (iPS), aimed at obtaining retinal pigmented epithelium and light-sensitive retinal rods in the aforementioned ocular diseases, with encouraging preclinical and clinical results. ***Conclusions:*** From a medical point of view, we must not forget that the transplanted retinal epithelium cells may cause tumours, since they have been obtained from Embryonic Stem cells, and may trigger immune rejection problems since they are heterologous. These considerations attest to the ethical uncertainty of the results of these clinical trials, but above all, it must be stressed that whenever Embryonic Stem cells are used, a human embryo must be destroyed to obtain them, which of course has objective ethical difficulties.

1. Introduction

This paper describes some preclinical experiments conducted with human stem cells, as well as the early studies in which this type of cell therapy was used.

Essentially though, we review earlier (1) and more recently published data (2) on the first clinical trial in which human stem cells were used for the treatment of eye diseases. The study included 18 patients: 9 with Stargardt's disease and another 9 with AMD.

A recent article published online in The Lancet describes how retinal epithelium cells were obtained using human embryonic stem (ES) cells and subsequently transplanted into nine patients over the age of 18 with Stargardt's macular dystrophy and a further nine patients with age-related macular degeneration (AMD) (2). The patients were followed-up for 22 months with the relevant ophthalmological examinations. The studies were recorded on the Clinical Trials.gov website (3).

This was a phase 1/2 clinical trial, so its aim was to evaluate the safety and tolerability of treatment, not the clinical results. There was no evidence of negative side effects or rejection of the transplanted cell tissue; some minor adverse effects found were attributed to the vitreoretinal surgery and immunosuppressive therapy. Although the objective was not to assess the clinical effects, the investigators reported that vision was recovered in ten patients (in the treated eye), remained unchanged in seven, and decreased in one, while vision in the patients' untreated eye remained similar to the baseline values.

The authors stated that this was the first time that an experiment like this had been conducted, with survival of the cell implant and possible biological activity of the transplanted stem cell progeny, concluding that retinal epithelium cells can be derived from human ES cells and safely used in ophthalmological medicine.

2. Background of the experiments discussed here

2.1. Early preclinical experiments with human embryonic stem cells

A thorough examination of this topic is beyond the scope of this article, so we will simply refer to some of what we consider to be the most significant facts related to the matter at hand.

In 2005, Laflame et al. showed for the first time (4), later confirmed in 2007 (5), that cardiomyocytes could be derived from human ES cells and used to improve cardiac function in infarcted rat hearts, which was an impetus to boost new research in cardiology (6), (7), (8), (9).

Even at that time, the difficulty arose of how to obtain sufficient cardiomyocytes to be able to repair cardiac lesions in larger animals, which could hinder the transfer of this experiment to human medicine. However, a technique has recently been developed that allows a large number of cardiomyocytes to be obtained from human ES cells to treat damaged primate hearts, which the authors consider could open the door to transferring these experiments to human medicine (10).

In 2008, it was also shown that skeletal muscle cells could be obtained from human ES cells, and that when these were transferred to mice with muscular dystrophy, there was improvement in the function of the damaged muscles, with no secondary tumours (11).

These experiments suggest the possibility of using this same type of cells in patients with muscular dystrophy, especially Duchenne muscular dystrophy.

There have also been various preclinical experiments with human ES cells in the field of neurology, as stated in PNAS (12), but up until then, complete functional integration of the neuronal cells produced had not been achieved. This now appears to have been accomplished, however, as described in the aforementioned PNAS article, in which the authors showed that human ES cell-derived neurons transplanted in mice were capable of establishing synaptic connections with the cortical neurons of the transplanted mice.

Another interesting article is published in Nature Biotechnology, in which a team from the University of Wisconsin-Madison in the United States transplanted human ES cells into mice with memory deficits. After the transplant these became nerve cells, which contributed to memory recovery in the sick mice (13). It is interesting to note that this was the first time that human ES cells were able to be implanted in animal brains and repair neurological impairments.

Another of the most promising experimental advances in the use of ES cells is in the area of ophthalmology. Thus, in a study published in Nature Biotechnology, it was shown that light-sensitive retinal rods (photoreceptor precursors) can be produced from mouse ES cells (14). Possibly more important though, is that the cells produced were able to integrate within the retinas of adult mice with various retinopathies. Following the transplant, there was functional improvement and the transplanted cells remained present three weeks after the graft, even establishing nerve connections with the existing retinal circuitry. There is no doubt that these experiments could contribute to the treatment of human retinal diseases, especially AMD, and indeed are already underway.

3. Early clinical trials with human embryonic stem cells

The first clinical trial with ES cells was proposed by the American company Geron, and was aimed at the treatment of spinal cord injuries (15). The company NovoCell also proposed to obtain insulin-producing cells from ES cells; likewise Mytogen, another American company, proposed producing retinal pigmented epithelium cells to treat patients with AMD. All three proposals were rejected by the United States Food and Drug Administration (FDA) as there were, in their opinion, insufficient previous experiments in animals (15).

However, on 23 January 2009, following a proposal from Geron, the FDA approved the first phase 1 clinical trial with ES cells aimed at treating patients with spinal cord injuries (16), (17). Eleven patients were to be included in the trial; the first entered in October 2009, and by mid-September 2011, four patients had already been included. The company Neuralstem was also authorised to use neural stem cells for the treatment of amyotrophic lateral sclerosis, while Stem Cells Inc. received approval to treat Pelizaeus-Merzbacher disease, a fatal brain disease that affects children, using human ES cells.

The company Advanced Cell Technology also proposed a second clinical trial using ES cells to treat Stargardt's macular dystrophy, a progressive disease that affects young people, and which may eventually cause blindness in adulthood, and a third trial to treat AMD (18).

Additionally, Advanced Cell Technology proposed the first clinical trial with ES cells outside the United States, in Moorfields Eye Hospital, London. This likewise hoped to treat 12 patients with Stargardt's macular dystrophy (19).

However, the trial proposed by Geron did not produce the expected results, so on 14 November 2011, the Californian company announced that the trial was being halted, ostensibly due to financial constraints, since according to them, they lost 65 million dollars in the first three quarters of 2011 (20). However, it seems that the underlying reason was basically that good clinical results were not obtained, since as they themselves reported "the treatments seem to be safe, although they have not yielded any improvement in spinal cord function" (20). One way or another though, the fact remains that the first clinical trial initiated with ES cells has been stopped.

Apart from the ethical difficulties inherent in these types of experiments (it must not be forgotten that

human embryos must be destroyed in order to obtain ES cells for transplant), there are also medical difficulties, among them the possibility that tumours may develop in the transplant patients (16), as well as the risk of immunological rejection, since the transplanted cells are heterologous. This concern has recently been voiced by two noted experts in this biomedical area, James Thomson and John Gearhart, who have stated that "there is a great concern among many of us about the possibility that some patients included in these studies could develop tumours, which would be an authentic disaster" (16).

4. Current clinical trials with embryonic stem cells

Certain media often report that numerous major clinical trials are being conducted with human ES cells, which does not exactly match the reality. To confirm this, one simply has to go to the Clinical Trials.gov website, which showed that in October 2011, there were 110,468 ongoing clinical trials worldwide, in 174 countries. Of these, 3,601 were with adult stem cells and 11 with ES cells. However, if we analyse these data in more detail, we can see that only two of these were using ES cells for therapeutic purposes (21). When we reassessed these data in October 2013, we found virtually the same results: there were 144,360 ongoing clinical trials at that time, in 185 countries, 4,451 of which were using adult stem cells and 15 using ES cells. As in the previous case, detailed evaluation of these trials revealed that only three were specifically designed for therapeutic purposes, two for treating AMD and a third for producing retinal pigmented epithelium for the treatment of patients with Stargardt's disease (22).

In other words, we believe we can unequivocally state that, at present, there are only three ongoing clinical trials using ES cells, two aimed at the treatment of AMD and a third at treating Stargardt's disease.

4.1. Current data

The first data on the use of human ES cells to treat various types of macular degeneration were published in February 2012 (1).

This clinical trial used retinal pigmented epithelium cells obtained from human ES cells, and its objective was to assess the safety and tolerability of this therapy. The paper presented the results of two patients, a 50-year-old with Stargardt's macular dystrophy and a 70-year-old with AMD. The therapy did not have any negative side effects, and four-months post-transplant, both patients reported somewhat improved vision.

The first results of the clinical trial in question have now been published (2). The retinal epithelium cells obtained from human ES cells were transplanted into 9 patients with Stargardt's disease and another 9 patients with AMD. Almost no negative adverse effects were detected.

Of the 18 patients included in the trial, ten had improved vision in the treated eye, there were no changes observed in seven patients, and vision worsened in one patient. The authors are therefore highly optimistic of the results obtained.

5. Research with hiPS cells

A study led by Yasuo Kurimoto of the Kobe City Medical Center General Hospital in Japan has recently been published, describing the implantation of retinal pigmented epithelium obtained from human induced pluripotent stem cells (hiPS cells) generated by cell reprogramming of the patient's own epithelial cells, with satisfactory results (23). Unlike the trials using human ES cells described above, in this case embryos were not needed (and therefore not destroyed) to obtain the stem cells. Instead, adult stem cells from the patient him or herself were used. These cells are subjected to a reprogramming process to transform them into hiPS cells, which in turn are differentiated into retinal pigmented epithelium cells and then implanted into the patient. The authors report absence of complications following the transplant.

However, this first human trial using cells derived from hiPS cells was suspended in March 2015 (24). The authors have decided not to treat a second patient after Japan's new regenerative medicine laws come into effect last November.

In addition they had identified genetic mutations in the second patient that were not detectable in the patient's original fibroblasts. So that, it is not definitely know whether reprogramming process induced the hiPS abnormalities, although hiPS often acquire mutations and epigenetic and chromosomal changes in cut (25).

6. Ethical assessment

In terms of reporting the results of the aforementioned clinical trials, some of the media are already talking about their success. Specifically, one Spanish newspaper reported that *"the work led by Robert Lanza, scientific director of Advanced Cell Technology, has not only demonstrated the safety of the therapeutic use of stem cells, but it has also obtained positive results in the treatment of two eye diseases, which are the leading cause of blindness in developed countries"* (26).

We consider this statement to be excessively optimistic, not to mention unfounded, since phase 1 and 2 clinical trials, as we said, are not aimed at evaluating clinical results, but on confirming the safety and tolerability of the drug or product used. Furthermore, as has already been described, of 18 patients, beneficial effects were found in ten, no effects in seven and negative effects in one, which does not appear to be sufficient medical evidence to confirm this claim. Moreover, in our opinion, the number of patients included in the trial is insufficient to properly assess its validity as a medical therapy.

We also consider it ethically questionable that results of uncompleted clinical trials are assumed to be reliable, since this can create unfounded expectations in patients who suffer from these serious diseases. As stated in Nature, "unethical procedures, exploitation and inflated promises, that is what generally makes the headlines - and so it is with regenerative medicine and stem cells. Media reports have left the impression that the research is rather dubious...Then there are the regular reports of companies that are exploiting vulnerable - and often seriously ill - patients with promises of expensive, but unproven, miracle cures" (27). This value judgement can be attributed to the clinical trials discussed here, aimed at treating AMD using retinal epithelium cells derived from human ES cells. Using new therapies in humans, and assuming them to be good, requires great caution, something that we are not sure has been given due consideration in the case in question.

Furthermore, on assessing the ethicality of the experiments published in The Lancet (1) (2), we must also bear in mind that the work is funded by the pharmaceutical company Advanced Cell Technology itself, of which Lanza is the scientific director, with the ethical implications that this may entail.

Additionally, from a medical point of view, we must not forget that, as mentioned previously, the transplanted retinal epithelium cells may cause tumours, since they have been obtained from ES cells, and may trigger immune rejection problems since they are heterologous. Both are aspects that must be examined when conducting the medical experiments discussed here.

All these considerations attest to the ethical uncertainty of the results of these clinical trials, but above all, it must be stressed that whenever ES cells are used, a human embryo must be destroyed to obtain them, which of course has objective ethical difficulties. Therefore, we ask ourselves if it would not be better to lend our support to other types of clinical trials, especially those in which adult stem cells or reprogrammed adult cells (hiPS cells) are used. In fact, it is research with these types of cells that is experiencing a major boom in the search for clinical procedures that can be applied in regenerative therapy today.

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