OPINION ARTICLE

The Non Revealed Intelligibility of Stem Cells

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From the beginning of the clinical phase in the year 2001 (1,2), considerable expectation was generated in regards to cellular implantation as an attempt to recover myocardium viability both in acute stages of the disease as well as chronic, Although its future can be watched with optimism, there was no coherence in its methodological development. The matter was dealt with in an "all or nothing" fashion in regards to the results, and it was spread in the news without considering the analysis of chained methodological guidelines with the purpose of being explained. The resource to regenerate tissue that nature uses needs to be revealed in its intelligibility at its fundamental stages, from basic research towards the applied one.

Until this moment there has not been research strategies which tried to suitably solve the primary question, **cellular implants modify viability in a fibrous scar?** Fundamentally, the acute ischemia model was selected, which represents an attractor that reunites too many variables of synchronous action, impossible to be assessed independently, which cloud the analysis of the use of bone marrow stem cells (e.g. reperfusion by angioplasty, spontaneous recirculation in the injured zone, collagenolytic activity of matrix metalloproteinase, cellular implantation). Heisenberg's *principle of uncertainty* fits here. While we perform an angioplasty with verified effectiveness in the recently ischemic zone, at the same time we do not know how much of the evolution was due to the stem cell. And while we use these cells, we do not know the portion of benefit that corresponds to natural repair due to reperfusion.

Hence, primarily, acute models that obviously can be more effective in showing positive results were used, before appealing to logical developments to reveal the reason of which we are applying. In fact, these models leave open the dilemma on the increase of the real and topographic viability. They focus the search on functional results that are far from the initial step of organic viability that should be demonstrated with the purest possible models, trying to move away from the uncertainty of improving the injured zone through several simultaneous procedures. We see in them accidental and additional effects, not the causes that produce them.

In the fibrotic chronic model, the strict analysis of the effectiveness must be related in their results to the basic proposed objective, which consists of observing the changes in the nonviable areas, metabolically inactive and not revascularized which were implanted with cells. In this aspect, studies that show benefits in the treated segments are fundamental, since these patients are concomitantly revascularized. This situation still represents a limit in this model, although they present only ischemia in a remote zone of the grafted scars.

In order to advance in this subject it is necessary to rely on a methodology within the ethic realm and that in addition sets fundamental standards in viability change of the treated segments and that includes the smaller number of variables possible to preserve the specificity of the analysis. Our strategy was based on the selection of patients with a viable strip of the left ventricle, suitable for revascularization solely of the anterior descendent artery without extracorporeal circulation, without viability or arteries suitable for surgery in the remaining segments and with prior infarctions that occurred over a period of more than six months to avoid the possibility of the spontaneous recovery. Hence, patients eligible for surgery were ethically selected and who secondarily were grafted with cells in a model which only variable that was far from the viability analysis was the revascularization of the anterior descendent. With this model the extracorporeal circulation was avoided as well as the multiple possibility of post-pump inflammatory syndrome, and multiple revascularizations. Another important fact in this analysis was to consider akinetic and diskinetic segments. The hypo kinetic ones, although treated with implants of mononuclear cells during the same operating act, were excluded from the analysis to avoid the possible beneficial effect of myocardial revascularization surgery on them. On the other hand, we know that over 10% of nonviable segments are expected to improve with isolated myocardial revascularization. In addition, the fibrous segments have less of 25% of viable cardiomyocites. Due to the fact that at least 50% of functionally viable myocites are needed for the revascularization to be successful, it is conceivable to hypothesize that a change in the viability of the diskinetic and akinetic segments can be due to cellular implants.

Again, we infer that in science it is more feasible to see the effects than the causes. Of the possible acute (acute ischemias) and chronic (fibrotic myocardia, expanded myocardiopathies, Chagas) models, it is fundamental to work in viability changes extensively with verification by means of SPECT, PET or NMR. The

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fundamental step in this matter is to obtain verification of a change in the viability of the segments involved. Possibly, *score* scales suitable for minimum changes instead of the present ones that establish the viability of segments too wide for the changes that stem cells can introduce should be used.

In relation to the clinical situation, we believe that the accumulated experience in this last lustrum has shown some aspects that envision an image waiting to be revealed.

We should be pleased with the partial and provisional despite of the tendency of men in requesting unity and coherence when visualizing a world which essential feature is diversity. In this sense, our own experience allows us to see, beyond all the mysteries proposed in this review, the following: (3)

a) Traces of therapeutic effectiveness both at the level of the patient's functional capacity as well as positive changes in myocardial viability. Hopefully (we have seen this situation clinically) these patients will be able to show in their evolution, due to tissue recovery, episodes of angina pectoris that they did not show previously (self experience with myoblasts). The segmented study of the heart in order to observe the evolution of each of these partitions has been mandatory for this analysis.
b) The best perspective of regeneration in those segments with non transmural infarct in relation to transmural.
c) **Implantation is useful in patients with dilated myocardiopathies and ventricular dysfunction?** The best results obtained with nontransmural segments imply to discern that there is a possibility of advancing on other practically unexplored myocardiopathies in the clinical phase, such as the idiopathic expansion and Chagas disease. In this regard, in eight dilated patients (five idiopathic, two Chagasic and one with lupus heart disease) followed up during 180 ± 110 days post stem cells implant via catheterization, a 2.5 functional class that went from ± 0.76 to 1.37 ± 0.52 ($p < 0.001$) was observed , obtaining an increase of the ejection fraction of 18.25 ± 6.86% to 27.75 ± 9.51% ($p < 0.01$), whereas the diastolic diameter of the left ventricle decreased from 70.86 ± 11.70 to 65.11 ± 9.78 ($p < 0.05$).
d) Comprehension on the fact that those hearts with chronic infarctions and a diastolic diameter greater than 70 mm do not have good evolution compared to those less expanded.
e) The necessity of repetition of the procedure by noninvasive procedures to complete the reverse remodeling.
f) In view of the accumulated experience to date, the question: **are endothelial stem cells capable of being differentiated in cardiomyocites in chronic infarction?** In this regard, the estimation capillary density has shown better results with CD34 than with AC133. Perhaps the difference found can be due to the time of the implant in relation to the infarct, since in acute cases increased capillary density has been verified. The consideration of a better perspective in early treatment, after an infarct, is logical and rational. It would benefit from a better signaling that occurs during ischemia with vectors such as cytokines, endothelial growth factor, stromatic factor-1, alpha-hypoxic factor-1. Hence, **why does it also improve in chronic infarcts, if there is no increased capillary density?** Several possibilities appear at this point: interference in the fibrotic scars, reactivation of resident stem cells, and retention of transplanted cells.

In regards to the use of G-SCF (growth factor of granulocytic colonies), the protocol of the University of Navarra uses it as inductor during five days and later by plasmapheresis through a column the cellular fraction AC133 is selected. In opposition to the exclusive use of G-SCF, methodology carried out by Zohlnhöfer et al, (4) we should say that it is not shown that the isolated inducer is more effective than the recruitment of the searched pro angiogenic cells, situation that ensures the necessary number of cells in the obtained concentration. There is no certainty either that these cells free in the circulation nest in the adequate place with the enough percentage to avoid the necessity of *in situ* positioning. Attempts have been made to rapidly advance in search of answers in clinical application. Efforts should be strengthened in projects that ride between basic and applied research. The work of Zohlnhöfer et al (4) evidences that the type of cell and pathway used play a role not yet satisfactory in its results where chaos in the methodology used for the different communications is influential and needs to be self organized.

In this acceleration for results, innovation and simplification, myoblasts were rapidly set aside in favor of the bone marrow cells. Their thirty microns of diameter have made them inconvenient for the intracoronary pathway; therefore, their use in acute therapy as pointed by the majority of the studies is not feasible. In our experience with myoblasts, after 33 ± 6.05 months follow up we have arrived at useful considerations. (5, 6) If we consider the segments involved and we divide them in transmural infarct, nontransmural infarct, ischemic and normal, over 68 segments worth studying (in the 4 surviving patients over the 5 original ones), we found a clear recession in the segments with transmural infarct and an increase in the nontransmural and ischemic segments. In 80% of the analyzed transmural segments an improvement in viability was verified, when the original affection decreased from 15 segments to 3 ($p < 0.005$). The analysis of the nontransmural segments

must be exhaustive. Although these globally increased from 7 to 10, those nontransmural segments originally registered pre surgery decreased from 7 to 2 (72%). It is possible to explain that the global increase of these nontransmural segments, each one of them analyzed by radio isotope, corresponded to segments included due to the advance of the disease as well as at the expense of the transmural, with fibrotic tissue clearly receding. In terms of clinical experience, with myoblasts we have seen a performance maintained through time. If we compare them with stem cells of the bone marrow at seven months of follow up, the percentage of segments with transmural lesion that improved was 46%, whereas in the nontransmural ones a 90% benefit was obtained. After one year the efficiency in the transmural was maintained (52%), whereas in the nontransmural a 69 % decrease in the efficiency was observed. **Do the bone marrow cells need a reinjection at this time?** In fact, in three patients we performed a second application at that time with bone marrow cells. **Which is the best access route?** Catheterization will be in the future the access of choice, for being less risky and due to the possibility of not having to repeat it according to the patient's needs. At present, its exclusive use is not cause for a study on the implications of placing the cell in the adequate segment for future follow up. Access by catheterization will be able to benefit in the future if in myocardial fibrotics and with the use of it inter-surgical implants in the obligatory segments, a significant viability change is shown. Until the present time, the transmural and nontransmural segments previously analyzed in a chronic model allows us to be objective in that acutely ischemic patients appear as the most suitable to obtain favorable results. The additional assessment performed on segments with transmural and nontransmural lesions implies a possible therapeutic conception, both in regards to the early use of cellular cardiac implant in the course of sub complete/complete infarcts as well as its use in dilated idiopathic myocardiopathy and in Chagas disease. The interpretation is that in dotted *tiger-like* myocardia, with a mosaic of viable and nonviable zones, the recovery capacity would be greater than in the necrotic model, formed with great dilation and extensive fibrous scars. In the study by Janssens et al, (7) a randomized, double blind study with stem cells implants in patients with myocardium infarct and ST elevation, it is necessary in the first place to explain ethical aspects. **Is it feasible that bone marrow be extracted to a group of patients who are subjected to cardiac catheterization with the purpose of instilling saline solution to the coronary arteries of patients with acute infarctions to form the witness group?** We believe that the health industry promotes the advance of science over the ethics at dangerous levels no matter how hard the authors argue that they find that this experience is crucial. If we carried out a technical analysis in this study, the enrolled population is of low risk, since 38% of the infarcts are located in the right coronary area, whereas the ejection fraction in these patients reaches 55%. With this ejection fraction it is not possible to expect significant changes. The cellular treatment within 24 hours of the acute infarct treated with angioplasty was carried out during the activity of metalloproteinases, which is not advisable for the survival of the injected cells. Although the work finds the improvement with cellular therapy uncertain, the treated group has shown a favorable effect on the remodeling of the infarcted area. The models of clinical development in acute infarcts show the necessity to establish the time interval between the acute event and the moment of the implant. This fact is related to the biochemical phenomena that occur at the injured area at the extra cellular matrix level. Therefore, matrix metalloproteinases increase (MMP-1) between days 3 and 7 after the acute myocardial infarction is observed, to decrease to normal values after day 14. This family of molecules has the function of favoring or inhibiting the degradation of the extra cellular matrix. Also, collagen volume increases from the third day of the event and continue to increase until day 21 to form the fibrotic scar. It is assumed that the appropriate time for cell implant is between days 7 and 14, when the space not yet has been completely replaced by the scaring collagen, which would limit the stretching of the bordering zones, unfavoring or slowing down remodeling process.

The study of Meyer et al (8) analyzes the BOOST clinical trial after 18 months. It was performed in patients to who, after an acute infarct, an infusion with bone marrow cells was injected. In the original work of that study published by Wollert et al, (9) the authors found a significant improvement in the cardiac function of the group treated with cells after 6 months. At 18 months, this new study does not find significant differences in the left ventricle ejection fraction: 3.1% of improvement in the control group and 5.9% in the implanted group, which agrees with our previous analysis regarding performance sustain. **Cellular selection plays any role?** Logically, in accordance with the above, it should be considered that myoblasts can be important and maybe they should not be rejected in the future in inter surgical implants (in conjunction with bone marrow) and that in addition the specific selection of the bone marrow chosen population (total, subpopulations or specific cells) is a matter that should be explained.

Clinical studies will have to be carried out with the aid of basic research if we wish to apprehend the non revealed intelligibility of nature in this issue of stem cells. (10)

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