Unambiguous Identification of Implanted Cells After Cellular Cardiomyoplasty: A Critical Issue

To the Editor:

In their article describing cell implantation in the myocardium of rats, Davani et al. stress the attention on integration of the transplanted syngenic mesenchymal progenitor cells within myocardial architecture and on their differentiation into smooth muscle and endothelial cells. Their conclusions are based on experimental work using 4',6-diamidino-2-phenylindole (DAPI) as a cell marker. They raise the legitimate concern of false-negative results resulting from the dilution of the marker on cell proliferation. We think, however, that the occurrence of false-positive is also a concern with this type of cell labeling.

Our group has conducted research on an autologous muscle cell grafting procedure in canine urethra as well as in ovine myocardium. To validate the best tools for non-genetic cell labeling, we performed in vitro and in vivo assessment of myocardium. To validate the best tools for non-genetic cell cell grafting procedure in canine urethra as well as in ovine myocardium. To validate the best tools for non-genetic cell cell grafting procedure in canine urethra as well as in ovine myocardium. To validate the best tools for non-genetic cell cell grafting procedure in canine urethra as well as in ovine myocardium. To validate the best tools for non-genetic cell labeling. We think, however, that the occurrence of false-negative cells resulting from the dilution of the marker on cell proliferation. We think, however, that the occurrence of false-positive is also a concern with this type of cell labeling.

In addition to these in vitro assays, we carried out skeletal muscle cell implantation in the urethra of a dog and harvested the grafted area 24 hours post-surgery. We found a pocket of DAPI-labeled cells (presumably the grafted muscle cells) within the urethral wall. Many of these cells looked necrotic. The outer muscle layer was intensely marked with DAPI. Because implanted cells could not have possibly had time to colonize the whole urethra and change their phenotype to smooth muscle cells, this seriously raises the issue of resident cell labeling with DAPI starting as early as 2 hours after the experiment.

In vivo implantation of skeletal muscle derived cells as follows: We implanted either live DAPI-labeled cells, destroyed DAPI-labeled cells, or free DAPI in sheep myocardium. The grafted areas were harvested 1 hour after implantation. We did observe the live grafted cells and intensely DAPI-labeled neighboring cardiomyocytes. We also found numerous marked resident cardiomyocytes in the destroyed DAPI-labeled cell or free DAPI assays. Small DAPI-labeled capillaries were observed as well.

We conclude that re-uptake of DAPI disqualifies the use of this reagent for accurate tracking of implanted cells. We think that only genetic labeling will permit unambiguous identification of the implanted cells and analysis of their fate.

N. Borenstein, DVM, MS
M. Hekmati, PhD
IMM Recherche (Centre d’Expérimentation et de Recherche Appliquée)
Paris, France
cera@imm.fr

P. Bruneval, MD
Service d’Anatomie-pathologie
Hôpital Européen Georges Pompidou

Paris, France
patrick.bruneval@h-egp.ap-hop-paris.fr

D. Montarras, PhD
Unité Génétique Moléculaire du Développement
Institut Pasteur
Paris, France
dmontarr@pasteur.fr

Acknowledgment

This work is supported by a grant from the Association Française contre les Myopathies and the Centre National de la Recherche Scientifique.


Response

We have recently demonstrated the plasticity of 4',6-diamidino-2-phenylindole (DAPI)-labeled mesenchymal stem cells (MSCs) in rats.1 Similar results have been documented in a murine model by Eliopoulos et al.2 However, our study is to our knowledge the first report for this species. The observations reported by Borenstein et al are not entirely new and have been previously discussed by Dorfman et al.3 We feel the following points are pertinent to this discussion:

(1) The in vitro study by Borenstein et al showed that the release of DAPI by dead cells and its re-uptake by live cells requires both a high concentration of DAPI and direct contact between cells. A high number of dead cells (30×10^6) were also required to mark only 1×10^6 live cells after 2 hours. Therefore, this would mean that if 1×10^6 DAPI-labeled cells (as used in our study) have released their DAPI, only 0.03 cells in the host myocardium would be stained.

(2) In the first in vivo study by Borenstein et al, the authors did not provide the phenotype of marked cells in the outer muscle layer. Importantly, Skuk et al have shown a high infiltration of immune cells (neutrophils, macrophages, and lymphocytes) in the donor-cell implanted area. The staining of these cells by DAPI can produce false-positive results.

(3) In the second in vivo study by Borenstein et al, their observations raise some questions: (A) Why did they analyze DAPI-labeled cells 1 hour after implantation and (B) why did they not provide any information about the fate of these cells in the long term? In the their experiments, Dorfman et al showed that the injection of free DAPI did not mark cardiomyocytes 7 days after injection. In addition, the authors did not provide information for either the number of viable cells injected or any quantification of the cardiomyocytes and capillaries labeled. In our study,1 we have observed that 1 month after the administration of 1×10^6 DAPI-labeled MSCs, 22% of capillaries and vessels contained individual DAPI-labeled cells in the whole of the infarct area. This distribution of DAPI-labeled cells supports the idea of colonization of vessels by MSCs and their subsequent division at the luminal face. In addition, because of size, more cells must be injected in sheep than in rats. Therefore, statistically there are more dead cells in the injected area and consequently a higher risk of false-positive labeling.
The data reported here do not allow disqualification of DAPI for cell labeling. They do show, however, that using high amounts of dead cells labeled with DAPI can induce false-positive results mainly in the first hours after implantation. For us, the release of DAPI by dead cells and its re-uptake by live cells is a minor phenomenon and probably could not be observed when a low quantity of cells is used, as in our study. Further long-term studies are necessary to disqualify DAPI for cell labeling. Meanwhile, DAPI labeling is actually used for tracking grafted cells and can provide useful information of the fate of grafted cells when its limitations are known.5

Siamak Davani, MD, PhD
Bernard Royer, PhD
Jean-Pierre Kantelip, MD, PhD
Laboratoire de Pharmacologie
Faculté de Médecine
Besançon, France

Aliette Marandin, PhD
Patrick Hervé, MD, PhD
EFS Bourgogne Franche-Comté
Besançon, France

Nursen Mersin, MD
Joseph-Philippe Etievent, MD, PhD
Service de Chirurgie Thoracique et Cardiovasculaire
Besançon, France

Bernadette Kantelip, MD, PhD
Service d’Anatomie Pathologie
Besançon, France

Unambiguous Identification of Implanted Cells After Cellular Cardiomyoplasty: A Critical Issue

N. Borenstein, M. Hekmati, P. Bruneval and D. Montarras

doi: 10.1161/01.CIR.0000127605.76159.A1

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2004 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/109/18/e209

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation is online at:
http://circ.ahajournals.org//subscriptions/