In the absence of a proven IV preparation, the proposal by Huxtable et al. for the use of saline hydration and oral NAC seems eminently sensible although, as they point out, evidence for the effectiveness of oral NAC when given immediately prior to contrast exposure is limited. A variety of other maneuvers may also reduce the incidence of RCIN, and we would suggest the following be included in any protocol:

1. The use of iso-osmolar contrast agents (2).
2. Minimization of the radiocontrast dose employed (3) (e.g., biplane imaging and echocardiography in place of contrast ventriculography).
3. Maximum 4-h fast prior to contrast exposure to prevent salt and water depletion.
4. Cessation of potentially nephrotoxic drugs prior to contrast exposure and reinstitution when renal function has been shown to be stable.
5. Assessment of renal function three days’ postprocedure.

We believe that awareness of RCIN will do much to reduce its impact.

The suggestion of a third arm to the study to compare oral and IV NAC is an interesting one. However, this was discounted during the design stage of our trial owing to the low incidence of RCIN in patients treated with oral NAC (approximately 2% [4]). The estimated number of patients required to show a difference or to prove equivalence between treatments would thus have been prohibitively large.

Finally, we emphasize that our study did not include emergency patients, as this would not have allowed randomization to the slower hydration arm of the protocol.

Christopher S. R. Baker, PhD, MRCP
Department of Cardiology
Charing Cross Hospital
Hammersmith Hospitals NHS Trust
Fulham Palace Road
London W6 8RF
United Kingdom
E-mail: cbaker@hhnt.org

Atholl Johnston, PhD, MRCPath
Laurence R. I. Baker, MD, FRCP
Charles J. Knight, MD, MRCP

NAD(P)H Oxidase in the Failing Human Heart

With great interest we read the recent JACC editorial comment by Warnholtz and Munzel (1) in which they emphasize the importance of NAD(P)H-derived reactive oxygen species in heart failure. This comment was for the greater part inspired by the interesting study by Heymes et al. (2) published in the same issue of JACC, which shows by immunohistochemistry that gp91phox is expressed in human cardiomyocytes.

In their editorial comment, Warnholtz and Munzel state that Heymes et al. (2) for the first time provide evidence of the activation and expression of the NAD(P)H oxidase in human cardiomyocytes. However, in March 2003 we already published a study in which we provided evidence for the expression of gp91phox (Nox2) in human cardiomyocytes (3). This was proven not only by immunohistochemistry but also by Western blot analysis on isolated human cardiomyocytes instead of total-tissue homogenates.

Although both studies describe the expression of Nox2 in human cardiomyocytes, there are some differences. For example, Heymes et al. (2) show by Western blot on total-tissue homogenates that there is no difference in Nox2 expression between failing and nonfailing hearts, whereas we demonstrated by immunohistochemistry that the number of Nox2-expressing cardiomyocytes within the infarction area is significantly increased after acute myocardial infarction (AMI). We have to keep in mind, however, that with AMI we have studied an acute phenomenon, whereas congestive heart failure (CHF) is a more or less chronic process. This might explain the interesting differences in the pattern of Nox2 expression between acute and chronic heart disease and could point to a different regulation of Nox2 expression in both phenomena.

The data of Heymes et al. (2) therefore, corroborate our own findings in that we both, using different antibodies, show that Nox2 is expressed in human cardiomyocytes. Their measured increase in NAD(P)H oxidase activity after CHF and our increased Nox2 expression after AMI emphasize the role of the NAD(P)H oxidase(s) in human cardiovascular pathophysiology. We completely agree with Warnholtz and Munzel that the possible co-expression of other Nox isoforms, the functional contribution of the cardiomyocyte-specific oxidases to the ROS-mediated effects observed in cardiac tissue homogenates, and the search for possibilities of pharmacological intervention are important issues that need to be addressed now.

P. A. J. Krijnen, MSc
VU University Medical Center
Department of Pathology
Room 0E16
De Boelelaan 1117
1007 MB Amsterdam
The Netherlands
E-mail: p.a.j.krijnen@vumc.nl

C. Meischl, MD, PhD
C. A. Visser, MD, PhD
C. E. Hack, MD, PhD
H. W. M. Niessen, MD, PhD
D. Roos, PhD
REFERENCES


REPLY

In our recent editorial (1) we commented on the expression and activity of the NAD(P)H oxidase subunit gp91phox isoform in the failing human heart reported by Heymes et al. (2). We also stated that this evidence was provided for the first time, not mentioning the article by Krijnen et al. (3), which was published in the March issue of the Journal of Clinical Pathology. One simple reason for that was that our editorial comment was already in press when the report from Krijnen et al. appeared. In that study the investigators describe the existence of Nox 2 (gp91phox) in normal human cardiomyocytes and an up-regulation of the expression as assessed by Western blotting technique and by immunohistochemistry in viable and jeopardized cardiomyocytes of freshly infarcted areas. Since this report was published in March, we agree that the study was indeed the first to comment on the expression of the NAD(P)H oxidase subunit Nox2 in human cardiomyocytes. Although the researchers have observed an increase in Nox2 in myocardial areas subject to acute myocardial infarction (AMI), one cannot really compare this situation with that of chronic congestive heart failure. Acute myocardial infarction is always accompanied by an acute and severe inflammatory response, and in the studies by Krijnen et al. no data were provided as to whether these patients had clinical signs of acute heart failure. Thus, the correspondent may agree that Heymes et al. (2) provided the first evidence for a lack of changes in the expression of Nox2 but an increase in the activity of the enzyme in patients with severe chronic congestive heart failure.

Thomas Munzel, MD
Universitätsklinikum Hamburg-Eppendorf
Medizinische Klinik III
Schwerpunkte Kardiologie und Angiologie
Martinistrasse 52
20246 Hamburg
Germany
E-mail: muenzel@uke.uni-hamburg.de

Ascan Warnholtz, MD

Ascan Warnholtz, MD

REFERENCES


REPLY

We thank Dr. Krijnen and colleagues for their interest in our report (1). The data, presented at the American Heart Association scientific sessions in 2002 (2), build upon prior experimental studies from our group and others (see Ref. 1). Although Krijnen et al. (3) discuss some parallels between our study and their recent interesting publication, several important differences should be emphasized.

The NAD(P)H oxidase is a multisubunit complex that requires not only a core catalytic Nox subunit but also several other components (p22phox, p47phox, p40phox, rac) for its function (4). Clear evidence of NAD(P)H oxidase expression and activity requires both the demonstration of multiple subunits and evidence of biochemical activity, as provided in our report (1). In addition, analysis of expression based solely on immunoblotting or immunohistochemistry is complicated by the fact that several Nox isoforms have recently been reported (4). Ideally, antibody-based methods should be complemented by RNA-based analyses (1). The best approach in experimental studies may be to employ gene-modified models (5).

With respect to NAD(P)H oxidase activation, this is recognized to occur through posttranslational modifications of one or more subunits (e.g., phosphorylation of p47phox) and/or altered expression levels (4). In our opinion, comments on mechanisms of activation of this complex enzyme based solely on qualitative analyses of Nox2 by immunohistochemistry are probably unwarranted. Our study found no change in overall tissue expression level of the four main oxidase subunits in end-stage chronic heart failure, whereas immunofluorescence studies indicated a translocation of p47phox to the cardiomyocyte sarcolemma where gp91phox was also present (1). Nevertheless, activation mechanisms of the oxidase remain speculative.

Finally, the condition we studied (chronic nonischemic congestive heart failure) (1) is quite distinct from the focus of Krijnen’s report (acute myocardial infarction) (3). The NADPH oxidase subunit expression, cellular localization, stimuli for activation, and activation mechanisms could well be significantly different among these conditions.

Ajay M. Shah, MD, FRCP, FAHA
Department of Cardiology
GKT School of Medicine
Bessemer Road
London SE5 9PJ
United Kingdom
E-mail: ajay.shah@kcl.ac.uk

Christophe Heymes, PhD

REFERENCES