Limited effects of post-ischemic NHE blockade on $[\text{Na}^+]_i$ and $\text{pHi}$ in rat hearts explain its lack of cardioprotection

Michiel Ten Hove$^{a,1}$, Cees J.A. Van Echteld$^b,*$

$^a$Interuniversity Cardiology Institute of the Netherlands, The Netherlands

$^b$Department of Cardiology, Heart Lung Center Utrecht, University Medical Center, Room G02.523, P.O. Box 85500, 3508 GA Utrecht, The Netherlands

Received 26 February 2003; received in revised form 4 July 2003; accepted 26 July 2003

Time for primary review 27 days

Abstract

Objective: Na$^+$/H$^+$ exchanger (NHE) blockade fails as reperfusion therapy in patients with acute myocardial infarction. In experimental studies, the reports on the efficacy of NHE blockade only during reperfusion are inconsistent. Differences in the severity of ischemia and in drug delivery may explain these inconsistencies. Little is known about the primary goal of post-ischemic NHE blockade, i.e. reduction of Na$^+$ overload. Methods: Isolated rat hearts were subjected either to 60 min of low flow (0.2 ml/min) ischemia or 25 min of zero flow ischemia. Hearts were reperfused with or without the selective NHE blocker cariporide added to the perfusate. $[\text{Na}^+]_i$ and pH$_i$ were measured with simultaneous $^{23}\text{Na}$ and $^{31}\text{P}$ NMR spectroscopy. Results: After 60 min of low flow ischemia $[\text{Na}^+]_i$ had risen to 424 $\pm$ 14% of baseline and pH$_i$ was 6.36 $\pm$ 0.03. After low flow ischemia $[\text{Na}^+]_i$ and pH$_i$ recovered similarly in treated and untreated hearts. Recovery of the rate pressure product (RPP) was poorly in both groups. After 25 min of zero flow ischemia $[\text{Na}^+]_i$ had risen to 279 $\pm$ 7% of baseline and pH$_i$ was 6.12 $\pm$ 0.02. NHE blockade after zero flow ischemia caused $[\text{Na}^+]_i$ to decrease during the first 30 s of reperfusion, followed by a partial and transient rise during the second 30 s. Untreated hearts showed a very small rise in $[\text{Na}^+]_i$, during the first minute. pH$_i$ recovered 30 s slower in cariporide treated hearts than in untreated hearts ($p < 0.05$). No effect of cariporide on RPP could be detected since RPP recovered fully in untreated hearts. The end diastolic pressure, however, was increased during reperfusion to a similar extent in both groups. Conclusion: The lack of cardioprotection under these specific conditions of zero flow and low flow ischemia can be explained by the fact that NHE blockade only resulted in a small and transient effect on $[\text{Na}^+]_i$ and pH$_i$.

© 2003 European Society of Cardiology. Published by Elsevier B.V. All rights reserved.

Keywords: Na/H-exchanger; NMR; Ischemia; Reperfusion; Intra/extracellular ions

1. Introduction

In patients with an acute myocardial infarction (MI) blockade of Na$^+$/H$^+$ exchanger (NHE) at the onset of reperfusion failed to improve clinical outcome [1]. In a study with a small sample size, post-ischemic administration of the NHE blocker cariporide did result in reduced infarct sizes [2]. In contrast, in a large-scale trial with the NHE blocker eniporide, post-ischemic administration did not reduce infarct size [3]. As recently discussed [1], the discrepancy between both studies is most likely the result of a chance finding in the study with cariporide due to the small sample size.

This raises the question why NHE blockade fails as reperfusion therapy. As recently reviewed [4], many experimental studies show that blocking the NHE during ischemia is cardioprotective but reports on the efficacy of blocking the NHE only during reperfusion are inconsistent. In different species and models post-ischemic NHE blockade has been found to be protective [5–9] but other studies showed no effect [10–12]. However, when any residual flow is present during ischemia several studies report consistently that post-ischemic NHE blockade is ineffective [13,14]. Therefore, the efficacy of NHE blockade only during reperfusion may well depend on the nature of the ischemia, i.e. with or without residual flow.

Although the $[\text{Na}^+]_i$ is quickly reduced upon reperfusion after ischemia [15] it is likely that recovery of ischemic
acidosis mediates Na⁺ influx through the NHE. This NHE mediated Na⁺ influx may well be enhanced compared to the ischemic period due to the restored extracellular pH, resulting in a large transmembrane proton gradient. Therefore, it is likely that blocking the NHE upon reperfusion will reduce Na⁺ influx and thereby reversed Na⁺/Ca²⁺ exchange. However, the effect on the [Na⁺]i of NHE blockade with specific NHE blockers like cariporide (HOE 642) during reperfusion only never has been studied.

The purpose of this study was to find possible explanations for the failure of post-ischemic NHE blockade to offer cardioprotection in animal models, which may help to explain the poor clinical outcome in patients with acute MI treated with NHE blockers. The efficacy of post-ischemic NHE blockade may depend on the nature of the ischemia, which may vary within the group of patients with an MI. Therefore, we tested the effect of post-ischemic NHE blockade in isolated rat hearts subjected to either zero flow ischemia or low flow ischemia. To block the NHE, cariporide was administered immediately upon reperfusion (zero flow) or from the last 5 min of ischemia onwards (low flow). Simultaneous 31P and 23Na NMR spectroscopy was used to measure the pH_i, phosphocreatine (PCr), adenosine triphosphate (ATP), inorganic phosphate (Pi) and [Na⁺]i, respectively.

2. Methods

2.1. Heart perfusion

Twenty four Male Wistar rats were anesthetized with diethylether and hearts were perfused according to Langendorff as described before [16]. Briefly, hearts were perfused at 37 °C and a perfusion pressure of 73.5 mm Hg. Contractility was assessed by an intraventricular balloon and coronary flow was measured continuously. Hearts were paced at a constant voltage and at a frequency of 5 Hz throughout the protocol. The rate pressure product (RPP) (heart rate × LVDP) was used as an index of cardiac contractility. The investigation conforms with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). All perfusion fluids were saturated with 95% O₂/5% CO₂, resulting in a final perfuse pH of 7.35 ± 0.05. The standard perfusate contained (in mmol/l) Na⁺ 148.0; K⁺ 4.7; Ca²⁺ 1.3; Mg²⁺ 1.0; Cl⁻ 133.3; HCO₃⁻ 24.0; glucose 11.0. To discriminate between intra- and extracellular Na⁺, 3.5 mmol/l TmDOTP₅⁻ was used as a shift reagent, necessitating a lower [Ca²⁺]free of 0.85 mmol/l.

2.2. Experimental protocols

Two sets of experiments were performed. After stabilization, hearts were monitored during 5 min of control perfusion. Thereafter, hearts were either subjected to 25 min of zero flow ischemia or to 60 min of low flow ischemia (0.2 ml/min). In both cases hearts were reperfused for 30 min with a constant pressure. Hearts were reperfused with or without 3 μM cariporide (HOE 642) added to the perfusate. In the low flow group the drug was also administered during the last 5 min of low flow. To maintain gas composition of the perfusate all perfusion lines were surrounded by water gassed with 95% O₂/5% CO₂ to avoid gas leakage. Furthermore, during low flow hearts were perfused through a perfusion line with a small diameter (0.38 mm) to achieve a high flow velocity. After the protocol hearts were dried to determine dry weights. Intracellular volume was assumed to be 2.45 ml/g dry weight [17].

![Fig. 1. [Na⁺]i during 60 min low flow (0.2 ml/min) ischemia and 30 min reperfusion; hearts were untreated (solid diamonds) or treated with 3 μM cariporide from the last 5 min of low flow ischemia onwards (open squares) (n=6 for both groups); black bar indicates period of drug administration.](https://academic.oup.com/cardiovascres/article-abstract/61/3/522/404712)
2.3. NMR methods

As described before [16], $^{23}$Na and $^{31}$P NMR spectra were recorded simultaneously at 105.9 and 162.0 MHz, respectively, on a three channel Bruker Avance DRX400 spectrometer, equipped with a 9.4 T magnet, a dual tuned probehead and two digital receivers. Thirty-second $^{23}$Na NMR spectra were obtained by accumulation of 144 consecutive free induction decays (FIDs) using 90° pulses and a 210-ms interpulse delay. Thirty-second $^{31}$P spectra were acquired by adding 12 FIDs using 90° pulses and a 2.5-s interpulse delay. Parameters were quantified with a temporal resolution of 30 seconds, 1 or 5 min. Spectra were added accordingly. $^{23}$Na and $^{31}$P signals were quantified with respect to the signal intensity of a reference solution in a glass capillary, containing known amounts of Na$^+$ and methylene diphosphonate.

2.4. Calculations and statistics

pH$_i$ values were calculated from the chemical shift of the inorganic phosphate (Pi), as described before [16]. To quantify the speed of pH$_i$ recovery upon reperfusion the onset of pH$_i$ recovery was analyzed by determination of the time point at which pH$_i$ was increased by more then 0.1. We calculated the intracellular pH buffering capacity at the end of 25 min zero flow ischemia, using the intrinsic intracellular buffer capacity, $\beta_i$ [18], and we calculated the buffering capacity of the accumulated intracellular Pi and the CO$_2$ dependent buffering capacity assuming no CO$_2$ leakage nor CO$_2$ formation during zero flow ischemia. To estimate the Na$^+$ influx via the NHE.

![Fig. 2](image1.png)

Fig. 2. pH$_i$ during 60 min low flow (0.2 ml/min) ischemia and 30 min reperfusion with a temporal resolution of 5 min (A) and 30 s (B); hearts were untreated (solid diamonds) or treated with 3 mM cariporide from the last 5 min of low flow ischemia onwards (open squares) ($n=6$ for both groups); black bar indicates period of drug administration.

![Fig. 3](image2.png)

Fig. 3. Phosphocreatine (PCr) (A), adenosine triphosphate (ATP) (B) and inorganic phosphate (Pi) (C) during 60 min low flow (0.2 ml/min) ischemia and 30 min reperfusion; hearts were untreated (solid diamonds) or treated with 3 mM cariporide from the last 5 min of low flow ischemia onwards (open squares) ($n=6$ for both groups); black bar indicates period of drug administration.
during the first 30 s of reperfusion after 25 min of zero flow ischemia, we assumed that the difference in [Na⁺]i between treated and untreated hearts was solely the result of the absence of NHE activity in the cariporide treated hearts. Results are presented as mean ± S.E. Data were analyzed by oneway analysis of variance (ANOVA) with repeated measurements or with a Student’s t-test, when appropriate.

3. Results

Baseline parameters were identical in all groups. Average intracellular concentrations (mmol/l) amounted to 9.8 ± 0.4 for Na⁺, 19.2 ± 1.2 for PCr and 14.8 ± 0.7 for ATP.

3.1. Low flow ischemia

3.1.1. Intracellular Na⁺

During low flow ischemia the [Na⁺]i increased immediately and constantly in both groups (Fig. 1). The rate of rise was similar to the one found in hearts subjected to zero flow ischemia. After 60 min of low flow ischemia [Na⁺]i had risen to 424 ± 14% of baseline. Upon reperfusion the [Na⁺]i decreased partially in both untreated and treated hearts (NS).

3.1.2. Intracellular pH

During low flow ischemia the pHi slowly decreased to 6.25 after 20 min in both groups (Fig. 2A). During the last 40 min of low flow ischemia, the pHi gradually increased to 6.36. During reperfusion 31P spectra of all hearts showed split Pi peaks, indicating heterogeneous pHi recovery. This most likely represents heterogeneous reperfusion. Well perfused areas will show a recovery of the pHi, whereas poorly reperfused areas will not. The well reperfused areas showed a quick and complete recovery in both groups (NS) (Fig. 2B). Poorly reperfused areas in both groups showed no recovery of acidosis at all. Since the cariporide is only certainly present in well reperfused areas, only the most alkaline peak is represented in pH figures.

3.1.3. Energy related phosphates

PCr rapidly declined during ischemia followed by a fast partial recovery during reperfusion in both groups (Fig. 3A). ATP gradually decreased during ischemia and poorly recovered during reperfusion in both groups (Fig. 3B). Pi accumulated during ischemia followed by a fast reduction during reperfusion in both groups (Fig. 3C). Pi peaks were split in all hearts during reperfusion. In the cariporide treated hearts the peak representing the lowest pHi was relatively larger.
than in the untreated hearts (data not shown). Data on PCr, ATP and Pi showed no significant differences between both groups.

### 3.1.4. Contractile performance and coronary flow

During reperfusion the RPP recovered only partially in both groups (Fig. 4A). The recovery of the RPP tended to be better in untreated hearts but there were no significant differences. During reperfusion, heart rate did not follow in all hearts the pacing signal. During ischemia all hearts went into contracture. Characteristics of contracture did not differ between groups. As shown in Fig. 4B, the LVEDP increased upon reperfusion, followed by a gradual decrease in both groups (NS). During reperfusion the coronary flow was 58 ± 5% and 60 ± 4% of baseline in untreated and cariporide treated hearts, respectively (NS).

### 3.2. Zero flow ischemia

#### 3.2.1. Intracellular Na$^+$

During zero flow ischemia the [Na$^+$]$_i$ showed an immediate and constant increase as observed before [16] (Fig. 5A). Upon reperfusion the [Na$^+$]$_i$ in untreated hearts started to decrease gradually after 1 min reaching 140% of baseline values after 30 min of reperfusion. In hearts treated with cariporide the [Na$^+$]$_i$ showed an immediate fast decrease during the first 30 s of reperfusion (Fig. 5B), followed by a transient increase during the second 30 s. Thereafter [Na$^+$]$_i$ gradually decreased to a similar level as found in untreated hearts (Fig. 5A).

#### 3.2.2. Intracellular pH

During zero flow ischemia the pH$_i$ rapidly decreased in both groups to 6.1 after 25 min (Fig. 6A). pH$_i$ recovery differed, in contrast to the low flow experiments, between both groups. As shown in Fig. 6B, upon reperfusion the pH$_i$ in untreated hearts rapidly increased from the second period of 30 s onwards, whereas the pH$_i$ in cariporide...
3.2.3. Energy related phosphates

PCr decreased quickly during zero flow ischemia followed by a fast recovery during reperfusion in both groups (Fig. 7A). ATP gradually decreased during zero flow ischemia followed by a partial recovery during reperfusion (Fig. 7B). Pi accumulated during zero flow ischemia followed by a fast reduction during reperfusion (Fig. 7C). Data on PCr, ATP and Pi showed no significant differences between both groups.

3.2.4. Contractile performance and coronary flow

The RPP collapsed immediately during ischemia and completely recovered in both groups during reperfusion (NS) (Fig. 8A). During reperfusion, heart rate did not follow in all hearts the pacing signal. During ischemia all hearts went into contracture. Characteristics of contracture did not differ between groups. As shown in Fig. 8B, the LVEDP rapidly increased upon reperfusion followed by a gradual decrease in both groups (NS). During reperfusion the coronary flow was 81 ± 7% and 75 ± 6% of baseline in untreated and cariporide treated hearts, respectively (NS).

4. Discussion

To elucidate why NHE blockade fails as reperfusion therapy this study shows, for the first time, the effect of post-ischemic NHE blockade on its primary goal, i.e. prevention of [Na⁺]ᵢ overload, with a specific NHE blocker. After 60 min of low flow, we did not find any significant effect of post-ischemic NHE blockade on either of the measured parameters. After 25 min of zero flow ischemia there was only a small effect on the decrease in [Na⁺]ᵢ, and a small delay in pHᵢ recovery.

The different effects of NHE blockade after zero flow ischemia and after low flow ischemia may be explained by the difference in ischemic pHᵢ. During low flow the acidosis is less severe, [H⁺]ᵢ,free was 1.8 times higher after 25 min of zero flow ischemia than after 60 min of low flow ischemia. In addition, the transsarcolemmal Na⁺ gradient is smaller after 60 min of low flow ischemia, since [Na⁺]ᵢ is higher than after 25 min of zero flow ischemia. Therefore, it is likely that the NHE will mediate much less Na⁺ influx after low flow ischemia than after zero flow ischemia under these conditions. Since we found no effect of NHE blockade after low flow ischemia on [Na⁺]ᵢ and pHᵢ one would not expect a reduction in reversed Na⁺/Ca²⁺ exchange.

These results therefore explain the lack of an acute cardioprotective effect after a period of ischemia with residual flow, found in this study as well as in others [13,14], in spite of the fact that the residual coronary flow during ischemia was very small (1–2%).

In the low flow groups, cariporide treated hearts showed a trend towards a worse recovery of the RPP than untreated hearts. This effect, however, was not significant (p = 0.18). It could be related to areas in the heart with a low pHᵢ due to NHE blockade. As mentioned, we found multiplicity in the Pi peaks during reperfusion. In the cariporide treated hearts the Pi peak representing a low pHᵢ was relatively larger than in the untreated hearts, in spite of a slightly higher coronary flow. In well reperfused areas the pHᵢ recovers quickly, also in the presence of cariporide. It could be, however, that in poorly reperfused areas NHE blockade impairs pHᵢ recovery. This would have a negative inotropic effect.

Although not the purpose of this study, we found, surprisingly, that the rate of rise in [Na⁺]ᵢ in hearts subjected to low flow ischemia was the same as in hearts subjected to zero flow ischemia, in spite of the difference in ischemic pHᵢ.

Our results only provide limited information on the cardioprotective effect of NHE blockade after a period of ischemia without residual flow since the untreated hearts showed a complete recovery of the RPP. Cariporide, however, did not prevent the post-ischemic elevation of the LVEDP, indicating only limited or no cardioprotection in this situation. This, as well as the inconsistent data in the literature can be explained by our data on [Na⁺]ᵢ and on pHᵢ. On both parameters there was an effect, which proves that cariporide is active immediately, but the effect was only...
transient. The initial faster decrease in [Na\(^+\)], was partly cancelled out during the second period of 30 s and the delay in pH\(_i\) recovery was only 30 seconds. NHE blockade is thought to be cardioprotective via reduced reversed Na\(^+/\)Ca\(^{2+}\) exchange [4]. This effect could result from reduced Na\(^+\) influx as well as from delayed pH\(_i\) recovery, since a low pH\(_i\) may inhibit the Na/Ca\(^{2+}\) exchanger [19–21]. Our data show that the reduction in Na\(^+\) influx is only limited and transient. This mechanism would therefore prevent only a little reversed Na\(^+\)/Ca\(^{2+}\) exchange. Furthermore, the effect on the [Na\(^+\)]\(_i\), is mainly present during the first 30 s when the pH\(_i\) is still low, further abolishing the effect of the transiently lower [Na\(^+\)].

The immediate faster reduction in [Na\(^+\)], followed by a transient increase has been found before in our laboratory with the less specific NHE blocker EIPA [15]. In that same study it was shown that the Na\(^+\)/K\(^+\) ATPase is immediately active upon reperfusion. The initial faster reduction in [Na\(^+\)]\(_i\), can therefore be explained by Na\(^+\) efflux through the Na\(^+\)/K\(^+\) ATPase and the absence of additional Na\(^+\) influx via Na\(^+\)/H\(^+\) exchange. The concomitant rise in [Na\(^+\)], was explained by Na\(^+\) influx via the Na\(^+\)/HCO\(_3\) co-transporter, suggesting that post-ischemic NHE blockade may be more effective when accompanied by simultaneous blockade of Na\(^+\)/HCO\(_3\) co-transport.

This explains the data on [Na\(^+\)], but appears in conflict with the data on pH\(_i\). In untreated hearts the pH\(_i\) does not rise substantially during the first 30 s. This is in conflict with H\(^+\) efflux via the NHE. Also the rise in [Na\(^+\)], during the second period of 30 s of reperfusion in cariporide treated hearts is not accompanied by any change in pH\(_i\), which is in apparent conflict with enhanced Na\(^+\)/HCO\(_3\) co-transport. The delay in pH\(_i\) recovery compared to the Na\(^+\) influx may be explained by the fact that the pH\(_i\) is buffered, whereas the [Na\(^+\)]\(_i\) is not. Within the assumptions mentioned in the methods, we calculated that during the first 30 s of reperfusion Na\(^+\)/H\(^+\) exchange transports 4.5 μmol Na\(^+\) per ml cytosol in 30 s. This implies that the maximum increase in pH\(_i\) as a result of Na\(^+\)/H\(^+\) exchange during the first 30 s of reperfusion was 0.06, which is very close to the value of 0.05 we found in untreated hearts with our \(^{31}\)P data.

In the cariporide treated hearts [Na\(^+\)], decreased during the first 30 seconds of reperfusion by 4.3 μmol/ml cytosol. During the second period of 30 s [Na\(^+\)], increased by 2.5 μmol/ml cytosol. Therefore the nett Na\(^+\) influx is increased by 6.8 μmol/ml cytosol. When this difference would be solely the result of enhanced Na\(^+\)/HCO\(_3\) co-transport the maximum increase in pH\(_i\) during the second period of 30 s of reperfusion would be 0.09, assuming that all Na\(^+\)/HCO\(_3\) co-transport was electroneutral, whereas we found an increase of 0.05. However, when all Na\(^+\)/HCO\(_3\) co-transport is electrogenic with a stochiometry of 1.2 for Na\(^+\)/HCO\(_3\) the maximum increase in pH\(_i\) would be 0.18. Therefore, the increase in the [Na\(^+\)], during the second period of 30 s of reperfusion in the cariporide group can only partially be explained by Na\(^+\)/HCO\(_3\) co-transport.

An additional explanation for the delay in pH\(_i\) recovery compared to the Na\(^+\) influx may be intracellular heterogeneity. The [Na\(^+\)]\(_i\) signal in the \(^{23}\)Na spectrum arises from the total Na\(^+\). The pH\(_i\) signal is determined by the chemical shift of the Pi signal. An immediate subsarcolemmal decrease in [Na\(^+\)], will result in an immediate decrease in the Na\(^+\) signal. The chemical shift of Pi, however, may be compromised by a heterogeneous distribution of H\(^+\) and of Pi in the cell. When protons are transported over the sarcolemma the subsarcolemmal [H\(^+\)] decreases followed by a subcellular redistribution of protons. Therefore, the bulk Pi may experience a somewhat delayed decrease in [H\(^+\)] compared to the subsarcolemmal areas.

Although the small effects of cariporide administration on [Na\(^+\)], and pH\(_i\) indicate NHE inhibition, the blockade could be incomplete. However, previously we have shown that in this same model the same concentration of cariporide effectively inhibits ischemic Na\(^+\) overload [16]. Furthermore, the idea that the NHE is inhibited is supported by the finding that the effect on [Na\(^+\)], is similar as the one found previously using EIPA [15].

Our findings add to the ongoing debate on NHE activity during ischemia and reperfusion [9,22,23]. As recently reviewed [24], only studies using the fluorescent indicator SBFI to study [Na\(^+\)], find a limited rise in [Na\(^+\)], during ischemia and a fast and steep increase immediately upon reperfusion, whereas all \(^{23}\)Na NMR spectroscopy studies report a substantial increase in [Na\(^+\)], during ischemia similar to the one found in this study [15,16,25–29]. Avkiran et al. [23] convincingly argumented against the idea that the NHE is inhibited during ischemia, to which our previous results lend further support [16]. The spectral characteristics of SBFI and other fluorescent indicators like indo-1 are affected by pH\(_i\) and intracellular protein environment [30,31], which change during ischemia and reperfusion. Most studies using SBFI in intact hearts, however, use pH calibration in a protein free aqueous solution [9,30] or do not correct for pH changes at all [6,32]. When pH\(_i\) is normalized data on [Na\(^+\)], measured by NMR or SBFI can be remarkably similar. For example, Varadarajan et al. [32] reported a [Na\(^+\)], of ~24 mM after 3 min of reperfusion following 30 min of global ischemia in isolated guinea pig hearts, which is comparable to what we found in rat hearts at that timepoint [16]. In that same article it was stated that their [Na\(^+\)], measurements during ischemia may underestimate the actual [Na\(^+\)], by more than 50% during ischemia due to intracellular acidosis.

In conclusion, our data explain the limited protection of post-ischemic NHE blockade as reflected by the inconsistency in the literature as well as the lack of efficacy of post-ischemic NHE blockade found in a large scale clinical trial. After a period of zero flow ischemia there is only a transient faster decrease in the [Na\(^+\)], and the recovery of the pH\(_i\) is delayed only by 30 s. After a period of ischemia with residual flow there is no effect at all on the [Na\(^+\)], and the pH\(_i\), probably due to the less severe acidosis.
Acknowledgements

MtH was supported by The Netherlands Heart Foundation (Grant 98.141). Equipment was financed by The Netherlands Organization for Scientific Research (NWO, Grant 902-16-202), the Royal Netherlands Academy for Arts and Sciences (KNAW, Grant D96.695) and the Interuniversity Cardiology Institute of the Netherlands. Cariporide was kindly provided by Aventis Pharma GmbH (Frankfurt am Main, Germany).

References