Chapter 6

Progressive Left Ventricular Hypertrophy after Withdrawal of Long-term ACE Inhibition Following Experimental Myocardial Infarction.

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Abstract

Background: Although discontinuation of chronic ACE-I therapy after MI is common in clinical practice, some clinical studies reported an increased incidence of ischemia-related events after withdrawal. To further address this issue, we assessed hemodynamic, neurohormonal and vascular consequences of withdrawing long-term ACE inhibitor (ACE-I) treatment after experimental myocardial infarction (MI).

Methods: Rats were subjected to coronary ligation to induce MI, and received quinapril (15 mg/kg/day) from 2 weeks to 14 months post-MI. Subsequently, surviving rats were randomized to sacrifice at 0, 4, and 6 weeks after ACE-I withdrawal. Rats were studied for signs of heart failure, hemodynamics and cardiac function, neurohormones, and vascular endothelial function.

Results: After discontinuation of ACE-I treatment, plasma aldosterone levels increased between 0-4 weeks without further increment thereafter, suggesting persistent RAAS activation. Acetylcholine-induced aortic relaxation was impaired both at 4 and 6 weeks, indicating rapid and sustained development of endothelial vasodilator dysfunction after withdrawal. Moreover, 24% of the rats developed heart failure signs (edema, dyspnea), and 3 rats died, all within 4 weeks after withdrawal. Significantly increased N-ANP levels and lung weights at 4, but not at 6 weeks suggest a transient volume overload. Finally, LV/body weight ratios significantly increased between 0-4 as well as 4-6 weeks, indicating progressive LV hypertrophy.

Conclusions: The observed alterations after withdrawing long-term post-MI quinapril treatment in the present study may account for an increased risk for ischemic events. By that our findings highlight the potentially harmful effects associated with abrupt discontinuation of long-term post-MI ACE inhibition, and imply careful clinical consideration in this matter.
**Introduction**

Chronic activation of the RAAS is regarded as one of the major causes of progressive deterioration of left ventricular pump function after myocardial MI. Accordingly, ACE-I therapy after MI remains the mainstay to inhibit the progression to LV dilatation and heart failure. In contrast to the well-studied beneficial effects of ACE inhibition therapy itself, remarkably little has been published about the consequences of its withdrawal. ACE-I withdrawal often occurs in clinical practice, mostly due to intolerance (±10% of all patients)\(^2\). In the CATS trial, post-MI patients were randomized to captopril or placebo for 12 months, followed by 1-month placebo. The captopril-withdrawn patients showed a high incidence of ischemia-related events within one month after withdrawing treatment\(^3\). The exact mechanism behind this finding is unknown, but it may be explained by a rebound phenomenon. Withdrawal of several cardiovascular drugs, such as β-blockers\(^4\), nitrates\(^5\), and statins\(^6\) can cause pronounced rebound effects, requiring stepwise cessation of therapy. Therefore, the current study aimed to further investigate the consequences of withdrawing chronic ACE-I therapy in rats with MI. We hypothesized that the protective effects of ACE inhibitors on cardiac function and morphology, and endothelial function in post-MI treatment decline shortly after withdrawal.

**Methods**

**Study design**

The investigation conforms to 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), and the animal research committee of the University of Groningen approved the study protocol. The study is an extension of one of our previous study on long-term ACE inhibition after myocardial infarction\(^7\). Male Sprague Dawley rats (Harlan, Zeist, The Netherlands), weighing 280±25g were subjected to coronary ligation as previously described\(^8\). Briefly, rats were anesthetized with isoflurane 2.0-2.5% in oxygen, after which rats were intubated and ventilated with this gas mixture. Subsequently, a left-sided thoracotomy was performed and the left anterior descending coronary artery was occluded with a 6-0 silk ligature 1-2 mm after the bifurcation. Mortality within the first 24 hours after surgery was 51%. Two weeks after coronary ligation, rats were assigned to treatment with quinapril (QUI) in a dose of 15 mg kg\(^{-1}\) day\(^{-1}\), mixed through food. Body weights and food intake were measured, and concentrations of quinapril in the chow were adjusted weekly. The rats were housed in clear polyethylene cages (4-5 per cage). The rooms were temperature- (22° C) and humidity- (50%) controlled and had a 12h light-dark cycle. Treatment was maintained for 14 months. At the end of the follow-up period, none of the surviving rats showed overt signs of chronic heart failure (dyspnea, edema). Rats were randomly subjected to 0 (n=10), 4 (n=12) or 6 (n=6) weeks quinapril withdrawal, before rats were sacrificed for assessment of LV function, neurohormones, and endothelial function.
**LV function**

Rats were anaesthetized as described above, the carotid artery was cannulated and a pressure tip catheter (Micro-Tip 3French, Millar instruments Inc., Houston TX, USA) connected to a PC with appropriate software (Millar instruments, Germany) was advanced into the left ventricle and used for determination of heart rates, and left ventricular systolic and diastolic pressures (LVSP and LVEDP). As indices for global contraction and relaxation, we determined the maximal rates of increase and decrease in LVP (\(dP/dt_{\text{max}}\) and \(dP/dt_{\text{min}}\)), corrected for LV developed pressure. Subsequently, the catheter was retracted into the aortic arch, and arterial pressures were recorded.

**Neurohormone measurements**

After LV function measurements, arterial blood was collected, anti-coagulated with EDTA, and centrifuged at 4000 RPM for 10 minutes at 4 °C. Plasma was stored at -80 °C until assay. Plasma for ACE activity determination was collected separately, and not anti-coagulated with EDTA. ACE activity in plasma was determined according to the Hippuryl-His-Leu method, as has been described in detail before\(^8\).

For other plasma neurohormone measurements, samples were transported on dry ice to the Core Laboratory at the University Hospital Dijkzigt, Rotterdam, The Netherlands, where all measurements were performed as previously described\(^9\). Plasma renin activity (PRA) was measured by determining the amount of angiotensin I generated from angiotensinogen with an in-house radioimmunoassay. Concentrations of N-terminal atrial natriuretic peptide (N-ANP) and aldosterone in plasma were measured with commercially available radioimmunoassays from Biotop (Oulu, Finland) and DPC (Los Angeles, CA, USA), respectively.

**Tissue processing & histology**

Hearts were excised and rinsed with ice cold NaCl (0.3%). The atria and right ventricle were removed on ice for determination of left ventricular weights. The apical 1/3 part of the LV was cut off, scar tissue and spared myocardium were separated and snap frozen in liquid nitrogen for measurement of ACE activity in the spared myocardium. A midventricular slice of the LV was fixated in 2% paraformaldehyde for histological analysis. Infarct size was determined by histology according to methods described before\(^10\). In brief, the sections were embedded in paraffin, and 5 µm slices were cut and stained with Sirius red/Fast green. Infarct size was determined as the percentage of scar to total LV circumference. The midventricular section provides adequate estimation of total left ventricular infarct size\(^11\). Only rats with MI sizes exceeding 20% of LV were included in analysis, since smaller infarcts are hemodynamically fully compensated in this model\(^12,13\). As an index of LV dilatation midventricular LV cavity areas were measured by planimetry. For quantification of cardiac fibrosis, left ventricular collagen volume fraction was measured by dividing Sirius Red-positive area by total myocardial area within a given field\(^14\). Per rat, 10 subendocardial fields were analyzed; the collagen-rich infarct-border and perivascular zones were not analyzed.
Endothelial function
Aorta segments were used to assess endothelial function, as previously described. After excision of the heart, the thoracic aorta was removed, immediately cleaned of adhering tissue, and cut into rings of 2 mm in length. Subsequently, rings were mounted on a contraction set-up connected to an isotonic displacement transducer. The aorta sections were placed in an organ bath filled with Krebs buffer (containing in mmol/L: NaCl (120.4), KCl (5.9), CaCl$_2$ (2.5), MgCl$_2$ (1.2), glucose (11.5), and NaHCO$_3$ (25.0)). The organ baths were continuously gassed with 95% O$_2$/5% CO$_2$, and kept at a temperature of 37$^\circ$C. After a stabilization period of at least 30 minutes, rings were contracted with 60 mM/L KCl to check for viability. Again, rings were washed and stabilized. Subsequently, rings were precontracted with 1µmol/L phenylephrine (PE), and responses to increasing concentrations of acetylcholine (ACh, 10$^{-8}$ mol/L to 10$^{-4}$ mol/L) were determined. Finally, maximal endothelium-independent vasodilatation to the exogenous NO donor sodium nitrite was determined (SN, 10$^{-2}$ mol/L). This method provides a relevant measure of endothelial function.

Statistical methods
Data are presented as mean±SEM in case of normal distribution, otherwise in box plots. Groups were compared using one-way analysis of variances (ANOVA) with least squared differences post hoc analysis for multiple comparisons in case of normal distribution. When parameters were not normally distributed, differences were compared with a Kruskal-Wallis test followed by Mann-Whitney tests for individual group comparisons. Differences were considered significant at the level of 0.05 (two tailed).

Results
General characteristics
A total of 44 rats with MI>20% was subjected to quinapril treatment for 14 months, during which 13 rats (28%) died. None of the 31 rats alive after the treatment period showed overt signs of heart failure. Of these 31 rats, 10 were (randomly) sacrificed immediately to establish LV function and neurohormones under ACE-I therapy (0 weeks of withdrawal), and 21 rats were subjected to 4 or 6 weeks quinapril withdrawal. Five of the rats subjected to withdrawal developed signs of heart failure (edema, dyspnea), and 3 of those died prematurely (after 4, 6, and 26 days, respectively). At sacrifice, average MI sizes were similar in the 3 different groups (table 1). Similarly, body weights were not significantly different.

LV remodeling
Parameters of LV remodeling are summarized in figure 1. Withdrawal caused a marked progression of cardiac hypertrophy, as indicated by LV weights/body weight ratios. LV/body weight ratios were significantly increased after 4 weeks, and further increased significantly between 4 and 6 weeks of therapy withdrawal.
Table 1. Basal characteristics of sacrificed rats with MI after 62 weeks chronic quinapril therapy, or quinapril followed by 4 and 6 weeks therapy withdrawal.

<table>
<thead>
<tr>
<th></th>
<th>Chronic QUI (n=10)</th>
<th>4 weeks after QUI withdrawal (n=12)</th>
<th>6 weeks after QUI withdrawal (n=6)</th>
</tr>
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<tbody>
<tr>
<td>Body weight</td>
<td>492±19</td>
<td>523±27</td>
<td>478±26</td>
</tr>
<tr>
<td>Infarct size (% of LV)</td>
<td>34.0±2.3</td>
<td>31.8±1.5</td>
<td>30.0±3.2</td>
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No significant differences were observed. QUI; quinapril 15 mg/kg/day

We found no evidence for interstitial fibrosis; likely, up to 4 weeks after withdrawal, collagen deposition paralleled LV hypertrophy, resulting in unchanged collagen volume fractions whereas between 4 and 6 weeks collagen deposition was lagging behind hypertrophy, as indicated by reduced collagen volume fractions.

![Graph showing effects of quinapril withdrawal on cardiac remodeling](image)

Figure 1. Effects of 4 and 6 weeks quinapril withdrawal on cardiac remodeling in rats with myocardial infarction. *p<0.05 versus chronic QUI, **p<0.005 vs chronic QUI, † p<0.05 vs 4 weeks of withdrawal.
LV cavity areas, indicating LV dilatation, were significantly increased at 4 weeks, but normalized after 6 weeks of withdrawal (figure 1), suggesting a transient phase of volume overload.

Figure 2. LV function during chronic quinapril treatment and after 4 and 6 weeks of withdrawal in rats with myocardial infarction. *p<0.05 and ** p<0.005 versus chronic quinapril.

**LV function**
LV end-diastolic pressures tended to increase after 4 weeks and normalize at 6 weeks of withdrawal (figure 2). In parallel, plasma concentrations of N-ANP, representing atrial and left ventricular wall stretch, showed a significant increase after 4, but not 6 weeks of withdrawal (figure 3). Lung wet weights, indicating edema, followed the same time
pattern. The significant correlation between lung weights and N-ANP levels indicates formation of lung edema due to cardiac overload (figure 3).

Mean arterial blood pressures (MAPs) were low in the rats sacrificed during quinapril therapy, and 4 weeks of withdrawal caused a marked increase in MAP (figure 2), that was sustained until 6 weeks. This increase in mean arterial pressure was paralleled by increased maximum LV systolic pressures after 4 and 6 weeks of withdrawal. However, maximum rates of contraction and relaxation corrected for LV developed pressure (dPdt\text{max} and dPdt\text{min}, respectively) were significantly decreased after quinapril withdrawal. Heart rates had not changed significantly after both 4 and 6 weeks of withdrawal.

![Plasma [N-ANP] vs Lung weight](image1)

**Figure 3.** Circulating N-terminal A-type natriuretic peptide (N-ANP) levels during chronic quinapril therapy and effects of withdrawal. Right panel: significant correlation between N-ANP levels and lung weights, suggesting that lung wet weights indicate lung edema as a result of volume overload. Dotted lines indicate 95% confidence interval for correlation between N-ANP and lung weights. *p<0.05 versus chronic quinapril.

**Endothelial function**

Endothelial function was assessed by measuring responsiveness to acetylcholine in aorta rings placed in a contraction set-up. Dose-response curves show that acetylcholine-dependent relaxation was significantly impaired as early as 4 weeks after quinapril withdrawal (figure 4A), with no further deterioration occurring between 4 and 6 weeks.
Endothelium-independent relaxation was also impaired, as evidenced by a decreased maximum response to the exogenous NO donor sodium nitrite after both 4 and 6 weeks of withdrawal, which indicates declined vascular smooth muscle reactivity to NO (figure 4B).

**Figure 4.** Endothelial function in rats chronically treated with quinapril, and effects of 4 and 6 weeks of withdrawal. A) Dose-response curves to acetylcholine. B) Maximum endothelium-independent vasodilatation after the NO donor sodium nitrite. * p<0.05 versus chronic quinapril, ** p<0.005 vs chronic QUI, † p<0.05 vs 4 weeks of withdrawal. PE; phenylephrine (1 µmol/L).

**Renin-angiotensin-aldosterone system**

Plasma renin activity was markedly reduced after withdrawal (figure 5). LV ACE activity, but not plasma ACE activity tended to increase after withdrawal. Nonetheless, production of aldosterone, the end product of the RAAS cascade, was markedly increased after 4 weeks of therapy withdrawal. From 4 to 6 weeks, RAAS-parameters did not change.

**Discussion**

Although discontinuation of ACE inhibitor therapy after MI is common in clinical practice, consequences of withdrawing chronic therapy were hardly studied. We established the effects of withdrawing long-term quinapril treatment in rats with MI.

**Left ventricular remodeling**

In patients with chronic heart failure blood pressure was reported to rise quickly after captopril withdrawal\textsuperscript{17}. Accordingly, in the present study arterial blood pressure
increased to normotensive levels after 4 weeks quinapril withdrawal, and remained stable. LV systolic pressures showed the same pattern. These findings might paradoxically suggest improved cardiac performance after discontinuation of treatment. However, increased arterial blood pressures also impose an increased afterload to the already functionally impaired heart. Moreover, increased LV systolic function implies increased cardiac oxygen demand. Taken together, this could increase the risk for ischemic events after ACE-I withdrawal.\textsuperscript{18,19} Furthermore, (initially compensatory) LV hypertrophy was progressive in nature with further increase in LV mass between 4 and 6 weeks after withdrawal, whereas LV performance did not further increase in this period. Moreover, maximum rates of contraction and relaxation, corrected for LV developed pressure, were significantly decreased after both 4 and 6 weeks of withdrawal, suggesting impaired contractile function. Hence, progressive LV hypertrophy without concomitant improvement in LV function in the present study should be regarded as a maladaptation.

\begin{figure}
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\includegraphics[width=\textwidth]{figure5}
\caption{Circulating and cardiac renin angiotensin system in rats with myocardial infarction after 62 weeks chronic quinapril therapy, and the effects of 4 and 6 weeks of withdrawal. * \textit{p}<0.05 and ** \textit{p}<0.005 versus chronic quinapril.}
\end{figure}
The hypertrophic response observed after quinapril withdrawal may have been the direct result of increased arterial blood pressure, as well as increased local Angiotensin II (Ang II) formation. The latter seems well in line with the increased LV tissue ACE activity after quinapril withdrawal in the present study. Increased cardiac Ang II levels may also lead to interstitial fibrosis, which in its turn is associated with altered diastolic LV function. Therefore we checked for fibrosis by determining total interstitial collagen volume percentages. We did not observe an increase in collagen, but even a significant decrease between 4 and 6 weeks of withdrawal. As collagen was determined as percentage of cardiac tissue, interstitial collagen deposition likely kept pace with cardiomyocyte hypertrophy during the first 4 weeks, whereas from 4 to 6 weeks it was lagging behind, resulting in a relative dilution of collagen. This indicates that quinapril withdrawal did not result in fibrosis during the first 6 weeks after discontinuation of quinapril treatment.

**Transient volume overload**
Withdrawal was associated with signs indicative for increased cardiac preload, such as increased plasma N-ANP, LV cavity, LVEDP, and lung weight, at 4, but not at 6 weeks of withdrawal. We refer to this as (transient) volume overload. Accordingly, 5 of 21 rats (24%) developed signs of heart failure (edema, dyspnea), all within the first 4 weeks after withdrawal. In addition, all 3 deaths during the withdrawal phase occurred during these first 4 weeks.

Clinical data on the induction of a transient volume overload after ACE-I withdrawal in a setting of LV dysfunction are indefinite. A double-blind withdrawal study in patients with chronic heart failure (continued quinapril versus placebo) reported a gradual worsening of clinical status, starting at 4 to 6 weeks after withdrawal. However, many of these patients already showed volume overload symptoms while on quinapril. Furthermore results may have been obscured by diuretic treatment. Our findings seem more consistent with a sub study of SOLVD, in which LV chamber sizes rapidly increased to pre-treatment levels during a 3-week withdrawal period that was preceded by 33 months of active ACE-I treatment. This was paralleled by high incidence of major adverse events, including myocardial infarction and unstable angina.

Notably, LV filling pressures and N-ANP did not increase progressively, but normalized to some extent after 6 weeks withdrawal. The reason is not clear, but it may be associated with progressive LV hypertrophy between 4 and 6 weeks. The resulting increased mass of contractile LV may deal more adequately with increased cardiac workload after ACE-I withdrawal.

**Endothelial dysfunction**
Endothelial dysfunction with increased tendency for coronary vasospasm and acute coronary thrombotic processes is associated with an increased risk for ischemic events, and could well have contributed to the adverse events mentioned above. In agreement with this, endothelium-dependent relaxation of the aorta in response to acetylcholine was considerably impaired as soon as 4 weeks after withdrawal.

In this experimental model, MI generally does not result in clearly detectable (aortic) endothelial dysfunction before 8-10 weeks. The underlying mechanism is thought...
to include impaired NO activity through oxidative stress, mediated by activation of the RAAS after MI\textsuperscript{25}. The notion that profound endothelial dysfunction was already present 4 weeks after withdrawal may reflect rebound RAAS activation after withdrawal, resulting in accelerated endothelial dysfunction in the current study. Maximal endothelium-independent relaxation to the NO donor SN was impaired as well. This further underlines the role of increased vascular oxidative stress causing reduced activity of NO, regardless whether NO is from endogenous or exogenous origin. In accordance with this, angiotensin II infusion was reported to attenuate the response to nitroglycerin, which could be prevented when oxidative stress was reduced by co-treatment with superoxide dismutase\textsuperscript{26}.

**RAAS activation**

Ang II exerts a negative feedback on both renin\textsuperscript{27} and ACE\textsuperscript{28} expression. As a consequence, ACE-I-induced decreases in tissue Ang II levels result in markedly increased renin production\textsuperscript{29,30}. Accordingly, withdrawal of quinapril treatment could initially cause an overshoot in RAAS activity and blood pressure\textsuperscript{28}. In the current study, plasma ACE activity was unchanged after both 4 and 6 weeks. This could not be attributed to incomplete ACE inhibition at the time of withdrawal, as we showed previously that this dose results in optimal ACE inhibition\textsuperscript{7}. However, tissue -rather than circulating- ACE inhibition reflects effective ACE-I therapy\textsuperscript{8,31}. LV ACE activity tended to be similarly increased after 4 and 6 weeks of withdrawal. Moreover, plasma aldosterone had on average more than doubled, but to a similar extent after 4 and 6 weeks. Although rebound RAAS activation could have occurred within the first 4 weeks after withdrawal, as in this period mortality and signs of fluid retention were observed, our results suggest sustained rather than rebound activation of the renin-angiotensin- aldosterone system after withdrawal. The occurrence of a transient rebound effect after ACE-I withdrawal may depend on e.g. type and severity of underlying disease, ACE-I dosage, and duration of therapy before withdrawal\textsuperscript{32,33}.

**Study limitations**

The three groups were harvested at different time points after coronary ligation. This strategy allowed us to randomize rats individually to one of the three different groups, instead of per cage. This strategy could result in a confounding factor regarding the time course of heart failure development. However, the order of magnitude of difference in time point of sacrifice (weeks) is limited compared to the total treatment period (months). Moreover, comparison with MI-rats treated with the same dose of quinapril for 8 months showed that LV function and hypertrophy were hardly changing between 8 and 14 months\textsuperscript{7}, indicating that a time effect of heart failure development independent of quinapril withdrawal would be rather minor. We intended to sacrifice the withdrawal groups of rats at regular time intervals, i.e. 4 and 8 weeks. However, we observed clear manifestations of heart failure and a trend of increased mortality within the first weeks after withdrawal, and to anticipate that high mortality would lead to a very limited group size, we decided to sacrifice rats already after 6 weeks.
Samples for neurohormone measurements were obtained after invasive measurement of hemodynamics. This measurement of hemodynamics, as well as the anesthesia required for this procedure, could potentially interfere with neurohormone measurements. However, since all rats were treated according to the same procedure, and in general predictable differences were observed, reliable comparison between experimental groups seems feasible.

**Conclusion and clinical implications**

This study demonstrates that withdrawal of chronic ACE inhibition post-MI resulted in 1) progressive LV hypertrophy at persistent RAAS activation, 2) increased arterial blood pressures, 3) endothelial dysfunction, 4) a transient phase of volume overload early after withdrawal, in association with heart failure symptoms and increased mortality. Together, these processes may increase the risk for ischemic events in post-MI patients early after ACE-I withdrawal. Although extrapolation of animal data to patients has its difficulties, our data are consistent with the scarce clinical evidence of effects of ACE-I withdrawal\(^3,23\). Thus, abrupt discontinuation of post-MI ACE inhibition after long-term treatment should be considered very carefully, as it can be potentially harmful.

**References**
