Cardiorenal interaction
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Document Version
Publisher's PDF, also known as Version of record

Publication date:
2007

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):

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General discussion and Future perspectives
INTRODUCTION
Recent studies have disclosed a complex relationship between cardiovascular and renal disease. Chronic kidney disease is highly prevalent in patients with cardiovascular disease⁴, and moreover, cardiovascular risk is high in patients with renal dysfunction⁵,⁶. Recently, it has been shown that patients with only mild renal dysfunction have an increased risk to develop cardiovascular events⁷. Therapy resistance to ACEi and the vicious circle of the cardiorenal interaction result in an enormous clinical problem: not every patient with a decreased renal function will (equally) respond to the beneficial effects of ACEi⁸, and beside this they are more susceptible to suffer from a myocardial infarction. The aim of this thesis was therefore to get more insight in the mechanism behind the cardiorenal interaction and in the pharmacological approach to optimize therapy response to interfere in the negative spiral of the cardiorenal interaction.

CARDIORENAAL INTERACTION
Recently, enhanced progressive renal damage was described in a model of cardiorenal interaction elicited by myocardial infarction in unilaterally nephrectomized rats⁹. The mechanism behind this cardiorenal interaction is unclear, but candidate mechanisms, as hormone systems like the RAAS and the natriuretic peptide system, were hypothesized. In chapter 1 we confirmed this detrimental cardiorenal interaction and showed that it can be attenuated by treatment with an ACE-inhibitor with substantial beneficial effects on kidney and heart. ACEi therapy was effective in the prevention of enhanced renal damage caused by myocardial infarction since the level of proteinuria, interstitial and glomerular α-smooth muscle actin staining, glomerular surface area, and focal glomerulosclerosis incidence were significantly lower compared to the vehicle group. The level of renal inflammation (ED-1 staining) appeared to be significantly lower in the ACEi group. Interestingly, cardiac contractility and cardiac left ventricular end diastolic pressure showed a more favourable outcome in the ACEi compared to the vehicle group at the end of the study. In view of these findings, it is likely that the RAAS is of significant importance in this cardiorenal interaction model.

We showed that when cardiorenal damage was already present, RAAS intervention could prevent further progression of renal damage. Because in our experiment RAAS intervention was started at the time point renal disease had already developed, we do not know if development of renal damage after myocardial infarction would be present when RAAS intervention was already started directly after or even before myocardial infarction, at the time point when the RAAS is activated by the myocardial infarction. While RAAS intervention interferes with both renal and cardiac function, it is not known which part of the prevention of a further decrease in renal function is caused by a direct effect on the kidney or a more systemic effect on blood pressure or improvement of cardiac function. More specific treatment strategies for either the heart or the kidney have to be used in this model to clarify these questions. However, from clinical studies is already known that lowering microalbuminuria with an ACEi is cardioprotective⁷. This supports the hypothesis that RAAS stimulation is an important factor in the vicious circle between the heart and the kidneys.

In chapter 2 we investigated if ACEi intervention was effective in rats with severe renal and cardiac function loss to prevent further decline in renal and cardiac function. The main findings of this study are that the cardiorenal interaction model comprising of 5/6NX + MI features specific additional damage to both kidney and heart compared to 5/6NX or MI alone. Moreover, treatment with an ACEi
effectively attenuates these specific features of cardiorenal interaction in the rat. Beside the effects of ACEi we found no increase in renal damage in the nephrectomized rats with additive myocardial infarction: proteinuria, kidney weight, focal glomerulosclerosis, and interstitial α-SMA staining were comparable in 5/6NX and 5/6NX + MI. Blood pressure was comparable in both groups as well. However, creatinine clearance and renal blood flow were further reduced, which was improved by ACEi to levels comparable to isolated renal damage, as in 5/6NX alone. In the current study, ACEi therapy was very effective in 5/6NX and 5/6NX + MI in reversing the increase in hemodynamic parameters, like blood pressure, left ventricular peak systolic pressure, and cardiac hypertrophy. Moreover, it was effective in reducing renal damage i.e. proteinuria, focal glomerulosclerosis and interstitial α-SMA staining. Beside these comparable effects, ACEi therapy was able to restore the impaired creatinine clearance and renal blood flow in 5/6NX + MI to the levels in sole 5/6NX. These results are in favour of the important role of adjuvant RAAS stimulation after myocardial infarction to decrease renal function and renal blood flow. Whether the hypothesized vicious circle is present in rats with already sustained renal function loss experiencing a myocardial infarction did not become entirely clear. Renal damage did not increase any further compared to animals with sole renal function loss. However, a deterioration of renal hemodynamics was observed, which was responsive to ACEi therapy. This phenomenon could be explained by the RAAS activation after renal ablation by 5/6 nephrectomy: in animals with an already highly activated RAAS, an MI will not lead to more RAAS activation and therefore not to a further increase in renal damage. A plateau of damage might be reached. Beside this, the decrease in creatinine clearance and renal blood flow could be explained by a decrease in cardiac output after myocardial infarction and therefore renal perfusion as well. Although it is often thought that ACEi therapy will decrease renal function even more and ACEi is not always prescribed to patients with combined renal and heart failure8,9, our study indicates that especially after a cardiac event optimal therapy with an ACEi will improve renal hemodynamics.

Several interaction mechanisms were hypothesized to cause the cardiorenal interaction, like endothelial dysfunction, inflammation, reactive oxygen species and RAAS activation10,11. With respect to the heart, these mechanisms result in decreased cardiac output and cardiac remodeling as evidenced by left ventricular hypertrophy, interstitial fibrosis and a decreased capillary density. This process of cardiac remodeling shows similarities with features of cardiac aging. In chapter 3 we investigated whether cardiac remodeling due to renal function loss caused by 5/6NX is associated with cardiac telomere shortening, as a measure of damage representing accelerated aging. Telomeres form the end of chromosomes and prevent the loss of genetic information. In rats with severe renal failure we found shortening of telomeres in cardiac cells, while such a change was absent in rats with only mild renal function loss. The changes in cardiac telomere length in severe renal function loss were even comparable to the changes measured in rats that underwent myocardial infarction. The combination of severe renal damage and myocardial infarction did not lead to an excess in shortening of telomeres compared to sole 5/6NX and MI. From these results we conclude that in animals with renal failure, cardiac aging was present to a comparable extent as after myocardial infarction, although in animals with both renal and cardiac damage cardiac aging was comparable to aging in sole cardiac and renal damage. Apart from the absence of an increase in renal damage in animals with severe renal function loss after myocardial infarction, as described in chapter 2, we found that in these animals cardiac aging measured as telomere shortening was not increased as well. There are strong indications that oxidative stress plays
an essential role in the process of telomeric shortening\textsuperscript{12}. When in our model this mechanism behind telomeric shortening is present, both oxidative stress caused by 5/6 nephrectomy and by myocardial infarction induce the same amount of cardiac damage and therefore, in this model combined cardiac and renal damage did not result in an addition of individual damages.

**Therapeutic Perspectives**

Not every patient with chronic renal failure treated with an ACEi optimally benefits from this therapy\textsuperscript{6}, though to the large inter-individual variation in therapy response to these drugs which is only slightly modulated by differences in dose or class of drug\textsuperscript{13,14}. Because the amount of proteinuria reduction is correlated to renal prognosis\textsuperscript{15,16}, it is of the utmost importance to reduce proteinuria to the lowest possible level. Beside the RAAS, there are other peptide systems influencing in these disease processes, like the natriuretic peptide system, the vasopressin system, and the endothelin system. Insight into the effect of these peptide systems on the progression of renal damage could give insight in optimizing ACEi therapy.

In chapter\textsuperscript{1} we investigated the hypothesized additive effect of neutral endopeptidase inhibition (NEPi) to ACEi in a cardiorenal interaction model. In our previous study, we found a trend towards a decrease in natriuretic peptide levels in the group with combined cardiac and renal damage, while in the group with only cardiac damage higher levels were observed\textsuperscript{6}. From this observation, we hypothesized a role for natriuretic peptides in the deteriorating effect of cardiorenal interaction. However, no additional protective effect of VPI over ACEi on renal damage, measured as focal glomerulosclerosis, proteinuria, and interstitial and glomerular α-smooth muscle actin staining, was observed in the study. Although VPI was more effective than ACEi in prevention of podocyte damage, this did not result in the expected augmented prevention of increased proteinuria or focal glomerular sclerosis\textsuperscript{17}. Overall, this leaves only little evidence for a discernible beneficial effect of an increased level of natriuretic peptides beyond concurrent RAAS inhibition and associated blood pressure reduction in the presented cardiorenal model with short-term pharmacological intervention. We found that a VPI had no augmented effect on prevention of further renal and cardiac damage compared to ACEi, which could be explained by mechanism of action of the VPI. The rational to combine ACE and NEP inhibition is to potentate the antihypertensive actions of ACEi alone through vasodilator actions and diuretic and natriuretic actions. Because ACE and NEP are co-localized in many tissues, interaction between these two enzymes could be expected. Bradykinin metabolism is more inhibited leading to higher levels of bradykinin\textsuperscript{18}. In contrast, experiments in rats after myocardial infarction showed an increase in Ang II levels in plasma, aorta and lung\textsuperscript{19}. The increased levels of bradykinin could contribute to the natriuretic, hypotensive and cardioprotective effect of combined ACE and NEP inhibition, although the increased Ang II and decreased Ang (1-7) levels could counteract any benefit of ACEi depending on reduced Ang II and increased Ang (1-7) levels. Our data does however not exclude VPI’s to still have an important clinical contribution in both ‘renal’ and/or ‘cardiac’ patients, since VPI’s have proven to be effective in more isolated renal and cardiovascular disease in humans and rats\textsuperscript{20-23}.

Beside a combination therapy of ACE and NEP, inhibition of the natriuretic peptide system could be combined with inhibition of the endothelin system as well. Inhibition of the endothelin converting enzyme (ECEi) and NEPi has been proposed to lower proteinuria and therefore prevent further progression of renal damage in experimental renal disease\textsuperscript{24-28}. Although the effects on renal damage
of agents interfering with the endothelin system are controversial\textsuperscript{29,30}, therapy combining ACEi with ECE and/or NEP inhibition may optimize the antiproteinuric response. In chapter 4, we investigated the effects of ECE/NEPi in advanced renal damage after 5/6NX. We showed that ECE/NEPi did not affect the already developed proteinuria and focal glomerulosclerosis in this rat model for renal impairment. In contrast, ACEi effectively reduced proteinuria and prevented focal glomerulosclerosis. Beneficial cardiovascular effects of ECE/NEPi have been shown in different animal models of heart failure\textsuperscript{31}. Although after 5/6NX, the endothelin system is upregulated\textsuperscript{24,32} and cardiac hypertrophy develops due to hypertrophy of cardiomyocytes, expansion of interstitial tissue, arteriolar thickening and diminished capillary supply\textsuperscript{33,34}, we could not find improvement of cardiovascular parameters by ECE/NEPi within the time frame studied.

Likewise, there was no detectable effect of ECE/NEPi in advanced renal damage, while the endothelin system is activated in renal disease progress as well. In previous experiments, expression of endothelin-1 in the heart was increased after ligation of 5/6 of the renal tissue\textsuperscript{35}. Blockade of the endothelin A receptor prevented capillary/myocyte mismatch in rats after 5/6 nephrectomy, when treatment was started 24h after the operation\textsuperscript{16}. Beside the expression in the heart, urinary endothelin excretion was increased after 5/6NX\textsuperscript{37}. This implies that ECE/NEPi as inhibitor of the formation of endothelin could prevent the negative effects of endothelin-1 on the heart. After chronic infusion of endothelin, glomerular mesangial cell proliferation and constriction was observed providing arguments for an important role of endothelin in vascular reactivity and glomerular function\textsuperscript{38}. Endothelin-A and -B receptor antagonists have been shown to be effective in prevention of renal injury after 5/6NX when treatment was started 7 days after induction of the disease\textsuperscript{24,25,39}, although other studies could not confirm these beneficial effects\textsuperscript{29}. These results suggest that ECE/NEPi could be effective when supplied early in the development of the disease and therefore in prevention of renal and cardiac damage. Future studies must be performed to explore the effects of combining ECE/NEPi with ACEi in order to optimize therapy response to ACEi.

Another vasoactive peptide playing an important role in the pathogenesis of several cardiovascular diseases such as heart failure, hypertension, and chronic renal failure is vasopressin\textsuperscript{40,41}. Antagonizing vasopressin by a vasopressin receptor antagonist (VRA) has been shown to prevent the development of proteinuria and hypertension in the 5/6 nephrectomy model and proteinuria in the adriamycin nephrosis model\textsuperscript{42,43}. Because treatment of chronic renal failure is often initiated in patients when renal function loss is already present, in chapter 5 we compared early and late intervention with a V\textsubscript{1a}-receptor antagonist in the 5/6 nephrectomy model for renal damage. It was shown that the V\textsubscript{1a}-receptor antagonist protected against the early progression of renal injury caused by a reduction in nephron number, whereas its effectiveness seems limited in established renal damage caused by deteriorating nephron function of previously healthy nephrons. Although controversial, this is in line with the observation that vasopressin and the process of urine concentration might play a role in the loss of glomerular permeability selectivity in the hypertrophy of the remaining nephrons and in the hypertension observed early after 5/6 nephrectomy\textsuperscript{44,45}. In our study, a selective V\textsubscript{1a}-receptor antagonist was used. V\textsubscript{1a}-receptor antagonism might be useful in renal protection only in the prevention of renal damage in renal failure caused by an acute reduction in nephron number. From our study we can conclude that vasopressin might be important in the early pathogenesis of renal damage after 5/6NX by the V\textsubscript{1a}-receptor, although no sufficient evidence was provided, that in already sustained renal damage
vasopressin is important in further progression of renal disease, at least not via the \( V_{1a} \)-receptor. Additive NEP inhibition to ACEi was not superior over ACEi alone in prevention of further development of renal damage. Neither ECE/NEP inhibition nor VRA treatment showed to be effective in a model for sustained renal damage, while ACEi prevented further progression of renal damage. We can conclude that in the models for cardiac and renal damage we used, ACEi therapy is still superior. Therefore, optimizing antiproteinuric response should be considered in optimizing ACEi response itself. ACEi therapy resistance could be altered by kinetic or dynamic inter-individual variation. In chapter 6, we therefore investigated whether responsiveness to ACEi therapy on proteinuria in 5/6 nephrectomized rats is caused by a variation in kinetic or dynamic effects of the ACEi lisinopril. Thus far, the cause of the large inter-individual variation in response to ACEi therapy has not been thoroughly studied in rodent models of renal disease. In humans, it is known that the antiproteinuric effect of ACEi is dependent on sodium intake\textsuperscript{46}, ACE gene polymorphism\textsuperscript{5,47}, but probably not on initial proteinuria, blood pressure or GFR\textsuperscript{46}. Our study strengthens the hypothesis that the therapy response is not dependent on initial proteinuria and blood pressure. With respect to its pharmacodynamics, we found that high renal ACE activity might account for therapy resistance of ACEi. As the increased ACE activity in the non-responders was paralleled by an increase in renal protein levels of ACE, the difference is most likely explained by differences in the regulation of renal ACE expression. Tissue ACE expression is regulated by Angiotensin II levels via a negative feedback system\textsuperscript{48,49}. Possibly, the differential regulation of ACE expression in responders and non-responders is related to the ACE polymorphism in the rat\textsuperscript{50,51}. Previous studies have suggested that renal ACE expression governs the development of renal damage. In adriamycin nephrotic rats it was shown that the naturally occurring variation in baseline renal ACE activity in outbred animals predicts renal damage after the induction of disease\textsuperscript{52}. We have evidence that this is true for 5/6NX in outbred animals as well\textsuperscript{53}. As a pharmacokinetic cause we found a correlation between antiproteinuric response and inter individual variation in lisinopril excretion. It seems likely that a combination of pharmaco-dynamic and -kinetic variation could account for the inter-individual differences in antiproteinuric response in 5/6 nephrectomized animals. Because increased renal ACE expression could account for therapy resistance, optimizing ACEi therapy could be established by targeting ACEi to the exact location were renal ACE is localized: the brush boarders of the tubular cells. In chapter 7, we investigated whether an inferior response to ACEi under high sodium condition may be attenuated by targeting the drug to the kidney. Therefore, we studied the effect of captopril-lysozyme conjugate on adriamycin induced proteinuria in rats fed a high sodium diet. In that condition, captopril-lysozyme conjugate significantly reduced proteinuria without affecting blood pressure. In contrast, captopril treated animals displayed the opposite effect, \textit{i.e.} a reduction in blood pressure without any effect on proteinuria. Although captopril-lysozyme conjugate was administered in a dose five times lowers than captopril, even higher captopril levels were found in the kidneys. These results demonstrate the working profile of the captopril-lysozyme conjugate to be renoselective up to an extent where anti-proteinuric effects were observed, without any effect on blood pressure in nephrotic rats on high sodium diet.
General discussion

CONCLUSION

The aim of this thesis was to get more insight in the mechanism behind the cardiorenal interaction and in the pharmacological approach to optimize therapy response to interfere in the negative spiral of cardiorenal interaction. From the first part of this thesis we can conclude that in our cardiorenal interaction model renin angiotensin aldosterone system activation is an important mechanism leading to an increase in renal and probably also cardiac damage. Especially in animals with only mild renal function loss, RAAS activation could account for an increase in renal damage after myocardial infarction. In rats with already high plasma creatinine levels, inhibiting this RAAS activation with an ACEi showed to be effective in neutralizing this cardiorenal interaction: the increase in renal and cardiac damage caused by a myocardial infarction was counteracted by ACEi.

From the second part of this thesis we can conclude that in the animal models we used and in the time span in which therapy was applied, ACEi showed to be at least comparable or even preferable to the studied interventions in the other hormone/peptide systems. When renal damage was already established, ACEi prevented further progression of renal damage and reduced hypertension. ACE/NEPi showed comparable beneficial effects compared to ACEi. VRA and ECE/NEPi were not effective when therapy was started at the moment renal damage was already established, although VRA showed to be effective in prevention of further renal damage when therapy was started early in the development of the disease.

The beneficial therapy response to ACEi is highly variable between different individuals. This response variation could be explained by a difference in both pharmacodynamic and pharmacokinetic alterations of the ACEi. Especially variation in renal ACE activity, ACE expression and lisinopril clearance could account for the variation in therapy response to ACEi in rats. A strategy to optimize a blunted therapy response to ACEi by high sodium diet is to specifically target the ACEi to the kidney. We showed that chronic therapy with the renal selective captopril-lysozym-conjugate was more effective in reducing proteinuria than free captopril.

CLINICAL IMPLICATIONS

The results of this thesis may have clinical implications. Although prescribing of ACEi after myocardial infarction is already recommended in guidelines\textsuperscript{54}, refraining from adhering to these guidelines in patients with heart failure and renal function loss is reported\textsuperscript{9}. The more so, since the evidence for the cardiac protective qualities of RAAS-intervention are all obtained in patient population in which patient with renal dysfunction are excluded. In fact doctors refrain from treating cardiac patients with compromised renal function with an ACEi, since it often constitutes a problem with further (functional) falls in renal function in these patients. However, this thesis clearly shows that one would expect the biggest cardiac benefit in those with compromised renal function. In a post-hoc analysis of the RENAAL data, Remuzzi et al. indeed showed this to be happening in a clinical setting\textsuperscript{55}. The beneficial effects of ACEi on the development of renal damage after myocardial infarction (as a model for heart failure) in rats with mild and severe renal damage give evidence to change prescribing practice for patients experiencing a myocardial infarction into early use of ACEi, especially because pre-existing renal function is not always known. Data from the PREVEND study show that the prevalence of microalbuminuria in the general population is 5 to 7\%\textsuperscript{56}. Microalbuminuria is predictive for cardiovascular events: only a slightly increased level of albumin even in the normoalbuminuric range is related to an increased
cardiovascular risk\textsuperscript{57,58}. These individuals have more chance to experience a cardiovascular event and afterwards, cardiac function loss will lead to an increase in renal function loss as well. Breaking through this negative spiral with optimized ACEi therapy is of utmost importance.

**FUTURE PERSPECTIVES**

The cardiorenal interaction is assumed to be a very complex interaction of which we could only disentangle a small fragment. There are many players in the field, activated at different time points. Which adaptive mechanisms are exactly activated shortly after myocardial infarction and at what time point the most important negative influences on renal function occur is not known thus far. It is important to elucidate the total time frame of the cardiorenal interaction to investigate the most optimal moment to start the most optimal therapy.

In this thesis, it was shown that telomeric shortening is present in rats with renal function loss in a comparable amount as in rats with cardiac function loss. While oxidative stress might be present in kidneys with decreased cardiac output after a myocardial infarction, renal telomeric shortening could be present as well. However, whether an aging process is started in renal cells after myocardial infarction measured as telomeric shortening is not known yet. We were not able to measure oxidative stress and to test antioxidant treatment in our model, which would however be an elegant method to investigate the differences between the heart and the kidneys in contribution of oxidative stress in the cardiorenal interaction. While we hypothesize that RAAS activation is important in the cardiorenal interaction, it would be very interesting to investigate if RAAS intervention influences cardiac telomere length.

Optimizing ACE inhibitor therapy remains a hot research topic. Many patients worldwide benefit from this RAAS modulating group of drugs. Patents of classic ACEi, which proved to be very effective, expire resulting in a competition by the pharmaceutical industry to develop new drugs with new patents to secure their revenues. A lot of research is still necessary in both clinical and experimental settings to compare novel compounds with established ones, for which not much prescribing experience is available. Especially, by combining different vasoactive modulating methods, more antiproteinuric effects can be expected. For example compounds which inhibit the metallopeptidases ACE, ECE, and NEP are promising.

Thus far, every pharmacologic strategy in renal and cardiovascular disease exerts their effect on both heart and kidneys. This underscores the close relationship between these two organs, acting as one hemodynamic system. To discover more about this interacting system, it would be of special interest to selectively improve renal function in the cardiorenal interaction model to study the effect on cardiac function, and vice versa selectively improve the cardiac function. In this thesis we showed beneficial effect of captopril-lysozyme conjugate on the kidney without systemic blood pressure effects. Unfortunately, due to practical reasons this conjugate is not suitable to apply in high dosage in an experiment with substantial power. Other methods to specifically target a drug to the heart or the kidney should be explored. Given the current increase in genetically engineered drug targeting, this method could be very valuable in doing so.
REFERENCES


