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Mitigation of experimental radiation nephropathy by renin-equivalent doses of angiotensin converting enzyme inhibitors

John E. Moulder¹, Eric P. Cohen², and Brian L. Fish¹

¹Radiation Oncology, Medical College of Wisconsin, Milwaukee, Wisconsin, U.S.A.

²Staff physician, Medicine, Clement J. Zablocki Veterans Affairs Medical Center and Medical College of Wisconsin, Milwaukee, Wisconsin, U.S.A.

Abstract

Purpose—We tested five different angiotensin converting enzyme inhibitors (ACEI) as mitigators of experimental radiation nephropathy at drug doses calibrated to the plasma renin activity (PRA). This was done to determine whether all ACEI had the same efficacy as mitigators of radiation nephropathy when used at drug doses that gave equivalent suppression of the renin angiotensin system.

Method—10 Gy total body irradiation with bone marrow transplantation was used to cause radiation nephropathy in barrier-maintained rats. Equivalent ACEI doses were determined based on their effect to inhibit angiotensin converting enzyme (ACE) and raise the PRA in unirradiated animals.

Results—PRA-equivalent doses were found for captopril, lisinopril, enalapril, ramipril and fosinopril. These doses overlap the human doses of these drugs on a body surface area basis. All ACE inhibitors, except fosinopril, mitigated radiation nephropathy; captopril was a somewhat better mitigator than lisinopril, enalapril or ramipril.

Conclusions—Most, but not all, ACEI mitigate radiation nephropathy at doses that overlap their clinically-used doses (on a body surface area basis). Fosinopril is known to be an ineffective mitigator of radiation pneumonitis, and it also does not mitigate radiation nephropathy. These pre-clinical data are critical in planning human studies of the mitigation of normal tissue radiation injury.

Keywords

ACE inhibitors; radiation nephropathy; plasma renin activity; mitigation; total body irradiation; captopril

Corresponding author: John E. Moulder, Radiation Oncology, Medical College of Wisconsin, Milwaukee, Wisconsin, U.S.A. 53211, tel: 414-955-4672, fax: 414-955-6553, jmoulder@mcw.edu.

Declaration of Interest

The authors report no conflicts of interest.

Introduction

In 1986, Robbins and Hopewell (1986) reported that the angiotensin converting enzyme inhibitor (ACEI), captopril, mitigated the acute hemodynamic effects of renal irradiation in a porcine model. Since then a number of different ACEI have been shown to mitigate experimental late radiation injuries in tissues as diverse as kidney, lung and brain (Cohen et al., 2008; Moulder et al., 1998; Jenrow et al., 2010; Kma et al., 2012). There is also evidence for the efficacy of ACEI in mitigation of clinical radiation injuries to lung and kidney (Cohen et al., 2012), and in treatment of clinical radiation injuries to the kidney (Cohen et al., 2008). It is generally assumed that the ACEI are working via suppression of angiotensin II (ang II) production, although other mechanisms have not been conclusively eliminated (Cohen et al., 2010; Molteni et al., 2000). If ACEI do work by suppression of ang II production, then all ACEI should be equally effective if given at equivalent doses. In addition, one cannot assume that all ACEI will be interchangeable for any indication; preclinical studies are essential (Furberg and Pitt, 2001). We therefore tested the mitigation efficacy of a wide variety of ACEI at drug doses that caused equivalent suppression of the systemic renin angiotensin system. To define efficacy for suppression of the systemic renin angiotensin system we measured the effects of each ACEI on plasma renin activity (PRA). ACEI dose equivalence is critical, as the efficacy of each ACEI for inhibition of ACE in rats is quite different (Cushman et al., 1989b), and the efficacy of at least some ACEI as mitigators of radiation injury can vary tremendously with drug dose (Cohen et al., 1994). For assessment of efficacy for mitigation of radiation nephropathy, we measured the effect of each drug on radiation-induced renal morbidity (our primary endpoint) and on radiation-induced increases in blood urea nitrogen (BUN).

The choice of PRA as an index of the effect of ACE inhibitors on the systemic renin angiotensin system was based on the known effect of ACE inhibition to cause elevation of PRA (Ferguson et al., 1977). Equivalence of ACEI can be established by their effect of inhibiting ACE (Cushman et al., 1989a), which in turn leads to reduced ang II and to elevation of PRA via the known negative feedback mechanism (Brunner et al., 1972). The PRA effect does not depend on lowering of the arterial blood pressure (Ferguson et al., 1977). Alternative methods to establish equivalent ACEI doses would have been to measure plasma ang II or aldosterone; but the ang II assay requires special handling and the aldosterone levels may “break through” even with a constant dose of ACEI (Athtyros et al., 2007). PRA was also the choice over ang II or aldosterone measurements because the assay is robust and reproducible; all are critical issues in an experimental design that required hundreds of assays in experiments lasting for over two years.

BUN was chosen as a measure of radiation nephropathy because in irradiated rats it correlates closely with serum creatinine (Moulder et al., 1993), creatinine clearance (Cohen et al., 1994) and histopathological injury (Cohen et al., 1996), and it is predictive of renal morbidity (Moulder et al., 2011). Elevation in BUN in irradiated rats is also invariably accompanied by proteinuria and followed by hypertension (Cohen et al., 2002).

Materials and Methods

Experimental Design

In the first phase, normal rats were treated with five structurally different ACEI: captopril (containing a reducing thiol group), enalapril and ramipril (containing a dicarboxylate but no thiol group), fosinopril (containing a phosphonate but no thiol group) and lisinopril (containing a dicarboxylate but no thiol or phosphonate groups). Normal rats were treated with a range of doses of these ACEI, and PRA was measured after 10 days of continuous drug therapy. From the PRA data on unirradiated animals, the drug doses equivalent to 24 mg/kg/day of captopril (300 mg/l in drinking water) were calculated by parallel-line linear regression analysis of the log (drug dose) vs PRA relationship; see Figure 1 for an example of such a calculation and statistical methods for more detail. Then a second set of rats were given total body irradiation (TBI) at 10 Gy followed by a syngeneic bone marrow transplant (BMT) and treated with the ACEI at doses in the range that had been found to be equivalent in normal animals. Finally the third set of animals were treated with additional doses of enalapril and lisinopril in order to test the relationship of PRA to mitigation efficacy over a wider range of PRA. All irradiated animals were then followed until euthanasia criteria were reached, with assessment of BUN at intervals.

TBI was used because the research is based on developing mitigators for total body exposures such as those that could occur as a result of radiation accidents or radiological terrorism (Moulder, 2014; Moulder et al., 2011). In addition, we have previously shown that local renal irradiation and TBI cause essentially equivalent effects on renal function (Moulder et al., 1989), and that shielding the kidneys during TBI prevents all signs of renal injury (Lenarczyk et al., 2013). Drug therapy was started after TBI to avoid the possibility that some of the ACEI might be radioprotectors. ACEI therapy was started immediately after TBI and continued for the duration in order to maximize the ACEI effect, and make it more likely to detect differences in ACEI efficacy.

The Rat TBI/BMT Nephropathy Model

The studies were performed in syngeneic seven- to eight-week-old male WAG/RijCmcr rats that were bred and housed in a moderate security barrier. The rats were maintained in the Biomedical Resource Center of the Medical College of Wisconsin, which is fully accredited by the American Association of Accreditation of Laboratory Animal Care (AAALAC). The animal protocols were approved by the Institutional Animal Care and Use Committee (IACUC). The rat TBI/BMT nephropathy model is described in detail elsewhere (Moulder et al., 2011). In brief, a single-dose TBI regimen (10 Gy) plus BMT was used to cause radiation nephropathy that is characterized by proteinuria, azotemia and progressive hypertension that leads to renal failure as early as 15 weeks after TBI.

Irradiation was done with an XRAD 320KV orthovoltage x-ray system (PXI, North Branford, CT) operated at 320 kVp and 13 mAs with a half value layer of 1.4 mm Cu. During the irradiation, each unanesthetized rat was confined in a separate chamber in a plastic jig, which allows irradiation of 4 rats simultaneously. The 4 chambers were placed on a plane perpendicular to the beam direction with distance from source to the midline of rats

to set at 33.5cm. Collimator jaws were used to define a radiation field to be 22.5×22.5 cm² at midline and large enough to cover all 4 chambers with adequate (at least 2 cm) margins.

A Soft X-Ray Ionization Chamber (PTW-New York Corporation, Brooklyn, New York) was used to collect depth-dose information. Absolute calibration measurements were made using a Farmer-type ionization chamber and a Dose 1 electrometer (IBA Dosimetry GmbH, Schwarzenbruck Germany). This system was calibrated for the orthovoltage energy range at the Accredited Dosimetry Calibration Laboratory located at University of Wisconsin, Madison, WI. Measurements performed in this laboratory are directly traceable to the National Institute of Standards and Technology. The ionization was measured in air and then converted to absolute dose in water following the American Association of Physicists in Medicine Task Group-61 protocol (Ma et al., 2001). The dose rate for TBI was defined at the midline of the rat and was calculated to be 1.73 Gy/min. The total dose was delivered using one posterior-to-anterior beam. The dose at the centers of the 4 rat chambers varied by 2%, and rats were randomly assigned to chambers to avoid any resulting bias.

Within 2 hrs after TBI, rats received a BMT from a syngeneic donor (Moulder and Fish, 1989). Animals were monitored daily. Development of azotemia (as BUN) was assessed for up to 44 weeks after TBI, and animals that were morbid or whose BUN exceeded 120 mg/dl were euthanized. The BUN>120 mg/dl euthanasia criterion was based on negotiations with the IACUC (see detailed discussion elsewhere (Moulder et al., 2011)). The study was terminated after 44 weeks because the number of survivors was too low to justify further follow-up. BUN was determined with commercial kits (Cohen et al., 1994) at 9, 17, 21, 26, 31, 38 and 44 weeks.

ACEI Therapy and PRA Assay

Animals were provided with pharmaceutical grade captopril (Sigma Chemicals, St. Louis, MO, USA), enalapril (Sigma Chemicals), fosinopril (Sequoia Research Products Ltd., Pangbourne, UK) or lisinopril (21cEc PX Pharm Ltd, East Sussex, UK). These drugs were added to the drinking water as described elsewhere (Molteni et al., 2000; Kma et al., 2012; Moulder et al., 2011). Ramipril (21cEc PX Pharm Ltd) was given either in the drinking water (at 1.2 mg/l or lower) or in the food at higher doses; the high doses were given in the food (Harlan-Teklad base diet 8904, Madison, WI) as we needed doses where ramipril was not soluble in the drinking water. Table I shows the dose ranges that were tested. The drug doses were expressed in mg/m², assuming a baseline rat weight of 200 grams (Friereich et al., 1966). Use of the mg/m² dose expression enables a rational comparison of drug doses in experimental animals and humans (Reagan-Shaw et al., 2008).

PRA was measured by radioimmunoassay using a modification of the method of Sealey and Laragh (Sealey and Laragh, 1977).

Statistical Methods

Relative potency of drugs (Figure 1) were calculated using parallel line linear regression (Brownlee, 1965); this technique is mathematically equivalent to that used to derive hypoxic fractions from paired survival curve data (Moulder and Rockwell, 1984). Parallel dose response curves (log of the drug dose vs PRA) were fit to the raw data by linear regression,

and tested for goodness of fit by assessing whether there were deviations from linearity and parallelism. The horizontal difference between the parallel curves (log of the relative potency), and the 95% confidence interval on this difference were then derived from the linear regression analysis.

PRA and BUN values are shown as means with 95% confidence intervals. The time needed to reach a BUN of 120 mg/dl was estimated by a logarithmic interpolation between the last measurements above and below 120 mg/dl (Moulder et al., 2011). Time to reach BUN=120 mg/dl is shown as medians with 10–90% ranges. Survival curves were compared by the method of Lee and Desu (1972). Correlations of PRA with survival time and azotemia were done using the Kendall tau (τ) test (the non-parametric equivalent to the Pearson r test). The Kendall τ test was used in these analyses because it does not presume the nature of the relationship between parameters (e.g., it does not assume linearity), and because it can handle values that are out of range (i.e., animals that were still alive when the study was terminated).

Results

Plasma Renin Activity

In irradiated animals not receiving ACEI ($n=17$), PRA levels were 12.9 (10.0–15.8) ng/ml/hr at 10 days after TBI. In age-matched normal animals ($n=55$) the equivalent value was 10.8 (9.1–12.5) ng/ml/hr; this difference was not statistically significant ($p>0.20$).

ACEI treatment of normal animals raised PRA levels to double normal levels (Figures 1 and 2) after 10 days of drug treatment; measurements on a subset at 45 days did not show a significant further increase in PRA (data not shown). Above a threshold dose (which is different for each ACEI), PRA values were proportional to the log of the ACEI dose (Figures 1 and 2). PRA in irradiated animals receiving ACEI differed from that of unirradiated animals for some groups, but the differences were not statistically significant (data not shown). The lack of acute effects of renal irradiation on PRA has previously been shown (Cohen et al., 2002). Nevertheless, because PRA data for irradiated animals was sparse, determination of the relative potencies of the ACEI were based only on PRA in unirradiated animals.

Relative Potency of ACEI

Figure 1 and Table II show the relative potency of captopril and enalapril. The data for the other ACEI are shown in Figure 2 and Table II. Figure 2 shows average PRA values, but the actual analysis is based on the raw data. The relative potency of ACEI in these rats roughly parallels that in humans (last column in Table II vs last column in Table I).

Effects of Equal-Potency ACEI on Renal Morbidity after TBI

Doses of ACEI predicted (by the data in Table II) to be equivalent to 300 mg/l of captopril were tested in the radiation nephropathy model. The curves for time to development of morbidity are shown in Figure 3. Four of the five ACEI (captopril, enalapril, lisinopril, ramipril) were effective in delaying morbidity (all $p<0.05$), but fosinopril was not ($p>0.20$).

At doses required for equivalent PRA, lisinopril was as effective as captopril ($p=0.11$), but enalapril and ramipril were less effective ($p=0.05$), and fosinopril was considerably less effective ($p=0.03$). Interpretation of the differences between captopril and the others ACEI was complicated by the fact that at 10 days after TBI the PRA levels in the enalapril- and ramipril-treated animals were lower (means of 17 and 23 ng/ml/hr respectively) than in the other three ACEI (means of 28–31 ng/ml/hr).

To avoid a multiple comparisons problem, our experimental design was limited to two questions: were any of the ACEIs effective; and did any of them differ in efficacy from the “gold-standard” captopril. To respond to a reviewer’s question, we did a *post-hoc* analysis of whether there were significant differences between lisinopril, enalapril, ramipril and fosinopril (Figure 3). Without correction for multiple comparisons, fosinopril is less effective than lisinopril ($p=0.048$); none of the other comparisons show statistically-significant differences. With correction for multiple comparisons, none of these differences are statistically significant.

The mechanistic connection of ACE inhibition to mitigation of radiation injury was tested by comparing median times to morbidity with mean PRA levels measured 10 days after TBI in the same animals (Figure 4). This has the advantage of seeing the possible effects of small differences in PRA, and it allows us to include data for a lower dose of enalapril and higher doses of lisinopril. In general, PRA levels at 10 days after TBI are correlated with mitigation of renal morbidity; the exceptions are fosinopril, which is quite ineffective as a mitigator despite raising PRA, and captopril, which is somewhat more effective than predicted by its effect on PRA. The correlation of PRA with survival time was analyzed using the Kendall τ test. This test was used because time to morbidity was not known for animals that were terminated at the end of the experiment (at 44 weeks) with $BUN < 120$ mg/dl, and because we did not want to assume a linear relationship between PRA and time to morbidity. For all data, Kendall $\tau=0.51$ ($p=0.017$) showing a strong correlation of PRA at 10 days with survival. If the fosinopril data are not considered in the analysis, the correlation is even stronger ($\tau=0.67$, $p=0.004$).

Effects of Equal-Potency ACEI on Azotemia after TBI

In the animals treated with ACEI, BUN was assessed at intervals. The relationship between PRA at 10 days and BUN at 21 weeks is shown in Figure 5; 21 weeks was chosen for this analysis because it is the latest interval in which all animals on ACEI were still alive. PRA at 10 days is highly predictive of azotemia after 21 weeks for four of the five ACEI (captopril, enalapril, lisinopril, ramipril), but fosinopril shows much more azotemia at 21 weeks than expected from its effect on PRA. The correlation of PRA with BUN at 21 weeks was analyzed using the Kendall τ test. For all data Kendall $\tau=0.56$ ($p=0.010$) showing a strong correlation of PRA at 10 days with azotemia at 21 weeks. If the fosinopril data are not considered in the analysis, the correlation is even stronger ($\tau=0.67$, $p=0.004$).

Discussion

These new data confirm and extend our previous studies of the benefit of ACEI in mitigation of radiation nephropathy. In brief, previous work showed that a number of ACEI were

effective as mitigators of radiation nephropathy in rats, even when therapy was started weeks after irradiation and stopped as soon as 3–6 weeks later; and that they were effective regardless of whether the radiation was delivered locally or as TBI (Lenarczyk et al., 2013; Moulder et al., 2011; Cohen et al., 2010). Previous work also showed evidence for the efficacy of ACEI in mitigation and treatment of clinical radiation nephropathy (Cohen et al., 2012; Kharofa et al., 2012; Cohen et al., 2008). What the previous work did not show conclusively was whether ACEI are working via suppression of ang II production, as other mechanisms have been proposed (Cohen et al., 2010; Molteni et al., 2000).

This new work further supports the concept that comparative studies of ACEI, or any other mitigators, should be based on established markers of drug effect; and they should be done at doses that correspond to those used clinically, on a mg/m² basis. This new work also shows that inhibition of ACE does not ensure that an ACEI will be a significant mitigator of radiation injury. The choice of ACEI for clinical studies to assess the mitigation of radiation injuries must rely on pre-clinical evidence of efficacy.

There are clinical data on the dose-response relationship of ACEI to their therapeutic effect. Clinical use of ACEI as antihypertensive agents is dose-adjusted according to the level of control of the blood pressure, up to the upper limit of recommended doses. For treatment of subjects with kidney disease, some have advocated titration of the ACEI dose according to the degree of lowering of urinary protein (Cravedi et al., 2007), but such use is limited because excessive lowering of the blood pressure may increase mortality (Cushman et al., 2010; Kovesdy et al., 2013). In subjects with heart failure, many ACEI appear to be effective in reducing mortality (Garg and Yusuf, 1995), and higher rather than lower doses of ACEI are advocated (Pacher et al., 1996; Berger et al., 2000). But in none of these uses is ACE activity used to guide therapy.

The lack of benefit from fosinopril to mitigate radiation nephropathy remains unexplained. This ACE inhibitor also does not mitigate radiation pneumonitis (Kma et al., 2012). In addition to their action to inhibit ACE, ACEI also increase the activity of 11 β -hydroxysteroid dehydrogenase type 2, a key enzyme that attenuates the mineralocorticoid effect of corticosteroids by inactivating cortisol to corticosterone. That action could contribute to the antihypertensive effects of ACEI. Captopril enalapril, lisinopril and ramipril share this action, but fosinopril does not (Ricketts and Stewart, 1999; Riddle and McDaniel, 1994). We speculate that this could be one explanation for the lack of benefit of fosinopril in this model.

The renin-angiotensin system is not activated in experimental radiation nephropathy (Cohen et al., 2002), yet the degree of elevation of PRA caused by ACEI correlates well with the mitigation benefit in the present studies. This implicates the renin-angiotensin system as a key mechanism of injury in this model. An alternative explanation is that the effect of ACE inhibition is paralleled by effects of ACEI on pathways other than the renin-angiotensin system. That latter explanation is consistent with the discordant effects of fosinopril and captopril in the present studies; both inhibit ACE yet fosinopril has no mitigating effect and captopril's effect is better than expected.

The superiority of captopril in mitigating radiation nephropathy at doses that cause equal effect on the PRA confirms its status as the “gold-standard” ACEI for this use. We could speculate, as did Ward *et al.* (1989), that the sulfhydryl group might give captopril an edge by acting as an antioxidant. It also underlines the clinical imperative to consider that captopril remain a consideration for use in clinical trials that test the mitigation of radiation injury, even though it must be dosed up to three times a day. Indeed, we used captopril in our successful clinical trial of the mitigation of chronic kidney disease and radiation pneumonitis after hematopoietic stem cell transplantation (Cohen et al., 2012). ACEI with longer half-lives, including enalapril and lisinopril, are also effective mitigators of radiation injury in lung (Kharofa et al., 2012; Kma et al., 2012). Fosinopril mitigates neither radiation nephropathy (the present study) nor radiation pneumonitis (Kma et al., 2012). Thus, enalapril, and lisinopril can be considered for use in clinical trials testing mitigation of radiation injuries; and they will be easier for patients to use than captopril because they can be dosed once a day.

Importantly, the lack of efficacy of fosinopril emphasizes that a drug’s benefit on biomarkers of ACEI effect, such as blood pressure lowering and elevation of PRA, do not ensure that the drug will be a successful mitigator of radiation injury. Although it may not be the definite biomarker of the effect of ACEI, use of the PRA as a guide of ACEI effect may be useful for prospective clinical mitigation studies to confirm adherence to the study drug. In such studies, additional biomarkers could be tested to gain mechanistic insights. These could include serum activity of transforming growth factor beta, surfactant protein D, and Krebs von den lungen (KL-6) for lung injury (Graves et al., 2010) or urinary markers of injury in the case of kidney (Arthur et al., 2014). Successful mitigation by use of an ACEI in the presence of a significant effect on one biomarker, but not another, could identify that biomarker as a key mechanism of radiation injury.

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Figure 1.

Relationship between log of the drug dose (as mg/l in the drinking water and as mg/m²/day) and plasma renin activity (PRA) for captopril (●) vs. enalapril (○). PRA after 10 days on drug is plotted for individual unirradiated animals as a function of drug dose. Solid lines show parallel fits to the data (see Statistical Methods). The shaded area shows the 95% confidence interval for PRA in age-matched normal (unirradiated without drug) animals.

Figure 2. Relationship between log of the drug dose (as $\text{mg/m}^2/\text{day}$) and PRA for captopril (●), fosinopril (□, dotted line), lisinopril (◇, dot-dash line) and ramipril (△, dashed line). The curve for enalapril (from Fig 1) is shown as a gray line. PRA after 10 days on drug in unirradiated animals is plotted (means with 95% confidence intervals) as a function of drug dose. The lines for fosinopril, lisinopril and ramipril show parallel line fits to captopril; the fits are based on the raw data. The shaded area shows the 95% confidence interval for PRA in age-matched normal (unirradiated without drug) animals.

Figure 3.

Morbidity (BUN = 120 mg/l) incidence curves after 10 Gy TBI (plus BMT) for animals given ACEI at doses that caused equivalent elevation of PRA in normal animals that were on ACEI for 10 days. Drugs were given in the drinking water starting immediately after TBI and continuing for the duration. Data are shown for captopril at 300 mg/l (n=19), enalapril at 30 mg/l (n=9), fosinopril at 10 mg/l (n=8), lisinopril at 7–12 mg/l (n=14), and ramipril at 1.2 mg/l (n=9). Data are also shown for matched animals not given ACEI (n=16). Plotted incidence curves terminate at 44 weeks or when there were less than 2 animals still at risk.

Figure 4.

Time to morbidity (BUN = 120 mg/l) after 10 Gy TBI (plus BMT) as a function of PRA measured 10 days after TBI in the same animals. Data are shown for animals not given ACEI (X) and for animals given captopril (●), enalapril (○), fosinopril (□), lisinopril (◊) or ramipril (△). There were 14 animals not given ACEI, 17 given captopril, and 7–9 per group for the other ACEI. Drugs were given in the drinking water starting immediately after TBI and continuing for the duration, and the dose (in mg/l) is shown next to each point. Time to morbidity is shown as medians with 10–90% ranges; up arrows on the error bar indicate that >10% of animals were still alive (with BUN < 120 mg/l) at 44 weeks; for the two highest doses of lisinopril all animals still has BUN < 120 mg/dl at 44 weeks. The dashed line shows the linear trend of the data, but the actual analysis used the Kendall τ -test which does not assume linearity.

Figure 5.

Azotemia (as BUN) 21 weeks after 10 Gy TBI (plus BMT) as a function of PRA measured 10 days after TBI in the same animals. Data are shown for animals not given ACEI (X) and animals given captopril (●), enalapril (○), fosinopril (□), lisinopril (◇) or ramipril (△). Drugs were given in the drinking water starting immediately after TBI and continuing for the duration, and the dose (in mg/l) is shown next to each point. These are the same animals shown in Fig 4. BUN values are shown as means with 95% confidence intervals; and the shaded area shows BUN for normal age-matched animals. The dashed line shows the linear trend of the data, but the actual analysis used the Kendall τ -test which does not assume linearity.

TABLE I

Doses of ACEI Tested

Drug	Dose Tested in Rats ^a	Delivered Dose in Rats (mg/kg/day) ^b	Delivered Dose in Rats (mg/m ² /day) ^c	Dose Range Used in Humans (mg/m ² /day) ^d
Captopril	50–500 mg/l	4.2–42	28–275	3.9–175
Enalapril	5–60 mg/l	0.42–5.1	2.8–33	1.6–24
Fosinopril	5–100 mg/l	0.42–8.5	2.8–55	6.3–47
Lisinopril	2–30 mg/l	0.17–2.5	1.1–17	3.1–24
Ramipril	0.1–1.2 mg/l 1–5 mg/kg	0.0085–0.10 0.08–0.4	0.055–0.66 0.5–2.6	0.8–12

^a In water (when mg/l) or food (when mg/kg).

^b Assuming that a 200 g rat drinks 17 ml/day and eats 16 grams of food (as measured for these rats in our facility)

^c Body surface area data from Friereich *et al.* (1966).

^d Drug doses from Brown and Vaughan (1998) and body surface area assumed to be 1.6 m² Reagan-Shaw *et al.* (2008).

TABLE II

Relative Potencies of the ACEI Tested

Drug	Potency Relative to Captopril ^a	Dose Equivalent to 300 mg/l of Captopril (mg/l) ^b	Dose Equivalent to 300 mg/l of Captopril (mg/m ² /day) ^c
Captopril	--	--	165
Enalapril	11.9 (9.8–23.6)	25 (13–52)	14
Fosinopril	5.9 (2.8–10.2)	51 (30–106)	28
Lisinopril	29 (24–58)	10 (5–12)	6
Ramipril	270 (220–710)	1.1 (0.4–1.4)	0.61

^aRatio of ACEI doses giving equal effects on PRA. From analysis of the data in Figures 1 and 2, (with 95% confidence interval)

^bIn drinking water (with 95% confidence interval)

^cBest fit, with body surface area data from Friereich *et al.* (1966)