

**CENTER FOR DRUG EVALUATION AND
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PHARMACOLOGY REVIEW(S)

**DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH**

PHARMACOLOGY/TOXICOLOGY NDA/BLA REVIEW AND EVALUATION

Application number: 208686
Supporting document/s: Vasotec Tablet NDA 018998; Epaned Powder NDA
204308
Applicant's letter date: November 24, 2015
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Product: Enalapril maleate
Indications: Treatment of hypertension in adults and children
older than 1 month
Treatment of symptomatic heart failure in adults
Treatment of asymptomatic left ventricular
dysfunction in adults
Applicant: Silvergate Pharmaceuticals
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Any information or data necessary for approval of NDA 208686 that Silvergate Pharmaceuticals does not own or have a written right to reference constitutes one of the following: (1) published literature, or (2) a prior FDA finding of safety or effectiveness for a listed drug, as reflected in the drug product approved labeling.

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1 Executive Summary

1.1 Introduction

This NDA 208686 is to support a new formulation of enalapril maleate which is an angiotensin converting enzyme inhibitor (ACEi) for the treatment of hypertension in adults and children older than 1 month, heart failure in adults, and asymptomatic left ventricular dysfunction in adults. This is a ready to use oral solution of Epaned Powder titrated at 1 mg/mL mostly intended for a pediatric population. Vasotec Tablet and Epaned Powder have already been approved with a pediatric indication on their labels that was granted in 2001 for Vasotec Tablet and 2013 for Epaned Powder. The Epaned Powder pediatric indication is simply the Vasotec pediatric indication for the powder form of the drug. Vasotec Tablet and Epaned Powder were granted a pediatric indication in hypertension for children 1 month and older based on a PK study in children 2 months and older and before PREA was mandated so that no juvenile animal studies were required at the time to support the safety in the pediatric population.

This NDA is a 505(b)(2) application and as such new animal findings relevant to the indication for Epaned Oral Solution were derived from a comprehensive literature review that focused on the effects and safety of enalapril in the growing animals to complement the labeling information for the drug product. The focus on juvenile animals is justified due to the paucity of data in children in particular in neonates and infants treated with enalapril (pro-drug) or enalaprilat which is the active form of the drug product.

1.2 Brief Discussion of Non-clinical Findings from the Review of the Literature

A comprehensive review of the published studies in animals receiving the drug product (enalapril or enalaprilat) that spanned at least the last 15 years was used to discuss the non clinical findings. Margins of efficacy or safety (M) were calculated by comparing dosages used in the animals to the recommended starting (st) and maximum (m) daily dosages of enalapril maleate for a child or 0.08 mg/kg (st) and 0.02 mg/kg (m) and an adult or 40 mg (m), and 20 mg (m) in mg/M² equivalent orally and intravenously (IV), respectively.

Pharmacology and safety pharmacology in the adult animals:

Publications showed that enalapril prevented thrombosis in a rat model (M₃₀ that has not been observed clinically so far); reduction of ACE activity and elevation of ACE₂ might be one of the mechanisms underlying the antihypertensive function of enalapril in the rat; enalapril had a central nervous system antidepressant effect in the adult rat (M₂₅) and altered the EEG in the adult rabbit (M₁).

Toxicity in juvenile animals:

The majority of these studies of enalapril or enalaprilat used the juvenile rat and sometimes the piglet. Dosages were 20-500X the recommended pediatric dosages orally and IV in mg/M² equivalent. Authors justified these excessively high dosages in animals because “they have been

shown to block ACE in these models”. At these high dosages kidney, heart and lung toxicities, increased mortality, weight loss and failure to thrive were observed

Collectively the data showed that multiple administrations of enalapril or enalaprilat at supra-pharmacodynamic dosages in the neonatal period in intact rat pups and piglets resulted in irreversible kidney abnormalities and demonstrated that an intact renin angiotensin system may be necessary for normal kidney development during this period. Deleterious effects on heart and lung growth were also seen at these supra-pharmacological dosages. Multiple mechanisms and molecules seemed to be involved in the pathogenesis of the kidney abnormality at LD30% dosages, including kidney loss of TGF- β 1, activin-like kinase 1 (ALK-1), and ALK-5 activities, and aquaporine 2 protein expression, increased VEGF, and HO-1 protein expression. Absent testing at lower non-lethal dosage, it is not known to what extent the presence of any renal and other toxicity and pathogenesis might depend on the dose and timing of enalapril administration in the neonate animal. For example a very high dosage daily for 7 days relative to the recommended dosage in the rat pup (60 to 70 times the recommended oral dosage of 0.08 mg/kg in a child in mg/M² equivalent) upregulated a stress pathway consistent with ischemic kidney after enalapril exposure [Choi, 2005; Yin, 1997] whereas at a single lower dosage in a piglet study (about 10 times the recommended pediatric dosage of 0.02 mg/kg enalaprilat IV in mg/M²) there was no decrease in O₂ pressure in the kidney and the author hypothesized that enalapril exposure did not result in kidney ischemia but rather in “growth factor deficiency that might be responsible for the pathology observed” [Nilson, 2000].

Enalapril or enalaprilat showed lasting deleterious effects when administered even after nephrogenesis is normally completed in a neonatal rat model of perinatal Na⁺ overload and partial unilateral ureteral obstruction [Chen, 2007]. Again in these studies dosages were $\geq 60X$ the recommended pediatric dosages orally and IV in mg/M² equivalent. However the result observed in the model of partial ureteral obstruction was controversial because it was provoked during dosing in the 11 to 21 days post-natal period, beyond the critical 13 day post-natal window of susceptibility reported by Guron (1999) One must note that in another model of partial unilateral obstruction in the young weanling rat, enalapril exposure was beneficial at treating the condition at dosages close to the recommended pediatric dosages [Beharrie, 2004].

Overall conclusion from these studies:

Results of toxicity studies in juvenile animals demonstrated the susceptibility of the kidney to enalapril-induced toxicity during the 2-3 week post-natal period of completion of nephrogenesis, in the rat pup and the piglet and that receiving enalapril shortly after birth in the rat pup was nephrotoxic with kidney lesions and functional defects still being observed in the surviving adult rats. However, dosages in these studies were many multiple of the recommended dosages ($\geq 60X$) in the pediatric population and for the most part not verified at more clinically relevant dosages. Moreover, enalapril administration in weanling rats at dosages close to the recommended dosages in pediatric patients was actually beneficial in reversing the damage after chronic

unilateral ureteral obstruction, a condition that has been observed in the pediatric population. Because of both the excessive dosages used in the piglet and especially the rat pups and the differences in timing of organogenesis and maturation of kidney, lung, and heart, human neonatal risk is questionable. That said high dosages that were nephrotoxic in the 2-week post natal vulnerable period, were not nephrotoxic in the adult. Nephrogenesis in human is complete by 35 weeks of gestation and all functional nephrons are present at birth although tubular growth and differentiation proceed after birth. On the contrary in the rat nephrogenesis continues after birth until post-natal Day 14, a period of marked tubular growth and differentiation. Tubular differentiation in the pup rat continues until weaning at Day 21, and functional maturation is completed by 6 weeks of age.

Glomerular filtration rate in humans increases rapidly from birth to adult levels by 2 years of age while in the rat it rises sharply at birth until 6 weeks of age, when adult levels are reached. The kidney of a new-born rat is therefore comparable to that of a mid-trimester human fetus, a 14-day rat to an infant and a weaned rat to a 2-3 years old toddler.

Structural and functional development of the lung lasts up to 2 years in humans, whereas it is completed by 38-35 days in rats.

It is known that human fetal kidney function is compromised by maternal exposure to enalapril and other ACEi. Use of these agents in pregnancy can cause fetal anuria, resulting in oligohydramnios, and also lung hypoplasia, both of which which persist in the neonate [Hanssens, 1991]. The physiological hypotension at birth makes the neonatal kidney dependent on angiotensin II to maintain adequate GFR in the face of low mean arterial blood pressure ,and therefore even neonates not exposed to ACEi in utero are at high risk of renal dysfunction upon ACEi treatment [Lindle, 2013]. Also specific considerations should be given to premature births where the development of the kidney is retarded compared to that of a term baby. Indeed prematurity is a well-known risk factor for ACEi nephrotoxicity supported by case reports [Lee GJ, 2010]. Infants and neonates with pediatric cardiac diseases are also reported to be especially at risk of enalapril-induced kidney toxicity [Lindle, 2014; Nakamura, 1994].

1.3 Recommendations

1.3.1 Approvability

This NDA is approvable pending labeling changes.

1.3.2 Additional Non Clinical Recommendations

No non clinical recommendations are made. No attempt was made to alter plasma renin activity (PRA) in the neonate and juvenile animal studies, to inform whether risk of any enalapril- renal toxicity could depend on PRA.

1.3.3 Labeling

It is recommended that labelling be changed to reflect that neither efficacy nor safety of enalapril is known in the 1-month old newborn. See Product Information for details.

2 Drug Information

2.1 Drug

CAS Registry Number (Optional)

Generic Name

(b) (4) (1mg/mL)

Code Name

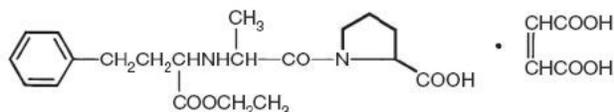
Chemical Name

Enalapril maleate

Molecular Formula/Molecular Weight

C₂₀H₂₈N₂O₅•C₄H₄O₄ / 492.52 daltons

Structure or Biochemical Description



Pharmacologic Class

Angiotensin converting enzyme inhibitor

2.2 Relevant INDs, NDAs, BLAs and DMFs

Vasotec Tablet NDA 018998; Epaned Powder NDA 204308.

2.3 Drug Formulation

The drug is an oral formulation of Epaned powder at 1mg/mL of enalapril maleate.

Components	Grade
Sodium benzoate	NF
Citric acid, (b) (4)	USP
Sodium citrate, (b) (4)	USP
Sucralose	NF
(b) (4) Mixed Berry Flavor, (b) (4)	In-house
Purified water	USP
Sodium hydroxide, (b) (4)	NF
(b) (4) hydrochloric acid, (b) (4)	NF

NF = National Formulary; USP = United States Pharmacopeia.

2.4 Comments on Novel Excipients

The formulation is using components, inactive ingredients and excipients in conformity with the NF, USP, and IIG for FDA approved products.

NF = National formulary;

USP = United States Pharmacopeia;

IIG = Inactive Ingredients database.

Excipient	Epaned Oral Solution		Approved Maximum Potency in Oral Liquid Form
	mg/mL	%w/v	
Sodium Benzoate, NF	(b) (4)		(b) (4)
Citric Acid, (b) (4) USP	(b) (4)		(b) (4)
Sodium Citrate, (b) (4) USP	(b) (4)		(b) (4)
Sucralose, NF	(b) (4)		(b) (4)
Sodium Hydroxide, NF, (b) (4)	pH adjustment	(b) (4)	(b) (4)
(b) (4) Hydrochloric Acid, NF, (b) (4)	pH adjustment	(b) (4)	(b) (4)
Purified Water, USP	(b) (4)	Not applicable	Not applicable

NF = National Formulary; qs = quantity sufficient; USP = United States Pharmacopeia.

All excipients are known to be GRAS substances at the maximum recommended dosages of the oral formulation.

2.5 Comments on Impurities/Degradants of Concern

There are no impurities and degradants of concerns. The specification for these is in accordance with the USP monograph for enalapril maleate tablets (Vasotec).

2.6 Proposed Clinical Population and Dosing Regimen

Epaned oral solution (1 mg/mL enalapril maleate) is for the treatment of:

- Hypertension in adults and children older than one month to lower blood pressure. Lowering blood pressure reduces the risk of fatal and nonfatal cardiovascular events, primarily strokes and myocardial infarctions;
- Treatment of symptomatic heart failure;
- Treatment of asymptomatic left ventricular dysfunction, to decrease the rate of development of overt heart failure and reduce hospitalization for heart failure.

Dosages:

- Hypertension indication: Adult: recommended initial dose is 5 mg once daily. Maximum dose is 40 mg daily. Pediatrics: recommended starting dose is 0.08 mg/kg (up to 5 mg) once daily;
- Heart failure indication: Initiate at 2.5 mg twice daily. Titrate up to 20 mg twice daily as tolerated.
- Asymptomatic left ventricular dysfunction indication: Initiate at 2.5 mg twice daily. Titrate up to 10 mg twice daily.

2.7 Regulatory Background

This a 505(b)(2) application. Vasotec® (enalapril maleate) 10-mg Oral Tablet (NDA 018998 initially approved December 24, 1985) is the RLD for Epaned Oral Solution. Sponsor cross-references to the non-clinical section of the Vasotec label to fulfill the nonclinical requirements for this 505(b)(2) application and to new non clinical literature information since the filing of NDA 018998.

3 Studies Not Reviewed

NA

The reader should note that the classification below is to facilitate reading. All articles were of an experimental nature and included aspects of pharmacology and/or toxicology.

4 Pharmacology

4.1 Primary Pharmacology

- Effects of enalapril on the expression of cardiac angiotensin-converting enzyme and angiotensin-converting enzyme 2 in spontaneously hypertensive rats. Zhen Yanga, et al. Arch Cardio Dis, 2013, 106:196—201.

The purpose of this study was to investigate the mRNA and protein expression of angiotensin converting enzyme 2 (ACE₂) and ACE in the heart from spontaneously hypertensive rats (SHRs) after enalapril administration.

Fifteen male SHRs, aged 16 weeks at the start of treatment received vehicle or enalapril at the dosage of 15 mg/kg/day (i.e. about 4 times the maximum daily oral dosage in an adult of 40 mg

daily in mg/M² equivalent) for 4 weeks. Ten Wistar Kyoto rats (WKYs) served as the normotensive control group, which were treated with vehicle.

Compared with in normotensive WKYs, the cardiac expression of ACE mRNA and protein in SHR was increased (1.68 ± 0.34 vs. 0.33 ± 0.12 , $p < 0.05$ and 1.21 ± 0.14 vs. 0.71 ± 0.11 , $p < 0.05$, respectively), whereas cardiac expression of ACE₂ mRNA and protein was decreased (0.50 ± 0.15 vs. 1.16 ± 0.24 , $p < 0.05$ and 0.71 ± 0.24 vs. 1.22 ± 0.14 , $p < 0.05$, respectively). After treatment with enalapril, the levels of ACE mRNA and protein were decreased (0.44 ± 0.19 vs. 1.68 ± 0.34 , $p < 0.01$ and 0.87 ± 0.13 vs. 1.21 ± 0.14 , $p < 0.05$, respectively), the level of ACE₂ mRNA was increased (1.77 ± 0.49 vs. 0.50 ± 0.15 , $p < 0.05$) but the level of ACE₂ protein remained unchanged.

The authors concluded that in SHR, the expression of cardiac ACE was remarkably increased, whereas ACE₂ was notably decreased and that the reduction of ACE and elevation of ACE₂ “might be one of the mechanisms underlying the antihypertensive function of enalapril in this SHR rat model.”

- ACE inhibition modulates transforming growth factor-beta receptors in the young rat. Nam Soo Kang, et al. *Pediatr Nephrol*, 2003, 18:865–871.

This study was designed to investigate the relationships between renal growth impairment and the protein expression of transforming growth factor-beta1 (TGF-beta1), TGF-beta receptor I (TbetaRI, activin-like kinase (ALK)-1 and ALK-5), and TGF-beta receptor II (TbetaRII).

Newborn rat pups were treated orally with enalapril at 30 mg/kg/day i.e. about 60 times the recommended pediatric starting oral dosage of 0.08 mg/kg in mg/M² or vehicle for 7 days.

Enalapril treatment resulted in an increased mortality (30%) by Day 7, and reduced body and kidney weights ($p < 0.05$ versus vehicle). Enalapril decreased renal TGF-beta1, ALK-1, and expression of ALK-5 protein and ALK-5 mRNA ($p < 0.05$), while TbetaRII expression was not changed.

The authors suggested that these results indicated that ACE inhibition in the developing kidney decreases TGF-beta1, ALK-1, and ALK-5 expression, which may account for renal growth impairment while TbetaRII may not be modulated by ACE inhibition in the developing kidney. However, it must be emphasized that this occurred at an approx LD_{30%}.

- Renal haemodynamics and function in weanling rats treated with enalapril from birth. Guron G. *Clin Exp Pharmacol Physiol*, 2005, 32(10):86570.

The aim of this study was to determine the consequences of neonatal angiotensin-converting enzyme (ACE) inhibition on renal haemodynamics and function in rats at 3-4 weeks of age. Male Wistar rat pups received intraperitoneal (ip) injections of enalaprilat 10 mg/kg/day i.e. about 80 to 100 times the maximum pediatric recommended dosage of 0.02 mg/kg/day IV in mg/M² or isotonic saline from birth until 24-28 days of age, when renal haemodynamics and function were assessed using clearance techniques under pentobarbital anaesthesia.

Enalapril-treated rats showed significant reductions in glomerular filtration rate (GFR; $-44 \pm 6\%$; $p < 0.05$), effective renal plasma flow (ERPF; $-33 \pm 6\%$; $p < 0.05$) and filtration fraction ($-16 \pm 3\%$; $p < 0.05$) compared with saline-treated controls. Although mean arterial pressure tended to be lower in enalapril-treated rats, this group demonstrated a significant increase in renal vascular resistance (RVR) compared with control rats (RVR; 46 ± 6 vs 32 ± 3 mmHg/mL per.min per g.kidney weight, respectively; $p < 0.05$). In enalapril-treated rats, urine osmolality was reduced ($-59 \pm 5\%$; $p < 0.05$) and urine flow rate and fractional urinary excretion rates of sodium and potassium were markedly elevated compared with controls ($p < 0.05$). Enalapril-treated rats showed severe renal histological abnormalities, including wall thickening of cortical arterioles, papillary atrophy and alterations of tubules in the interstitium. The author concluded that neonatal ACE inhibition in rats induces pronounced alterations in renal haemodynamics and function, characterized by reductions in ERPF and GFR, increased RVR and impaired tubular sodium and water reabsorption, which were evident at weaning.

Reviewer's comment: The excessive overdosage used has the potential for off-target effects, e.g. the atypically increased RVR.

- Mechanisms of impaired urinary concentrating ability in adult rats treated neonatally with enalapril. Guron G. Acta Physiol Scand., 1999, 165:103-112.

The aim of the present study was to determine the pathophysiological mechanisms underlying the defect in urine concentration in adult rats treated neonatally with enalapril. Male Wistar rats received daily ip of enalaprilat at the dosage of 10 mg/kg/day i.e. about 80 to 100 times the maximum pediatric recommended dosage of 0.02 mg/kg/day IV in mg/M² or vehicle (saline) from 3 to 24 days of age.

Assessments of fluid handling and maximal urine osmolality (Uosm(max)), renal function and tubular free water reabsorption (T(c)H₂O) under pentobarbital anaesthesia, renal tissue solute concentrations, renal aquaporin-2 (AQP2) expression, and kidney histology, were performed in 12-16-week-old rats.

Uosm(max) (1488 ± 109 vs. 2858 ± 116 mosm/kg, $p < 0.05$) and maximal T(c)H₂O were reduced in enalapril- vs. vehicle-treated rats after administration of 1-desamino-8-D-arginine vasopressin. Neonatally enalapril-treated rats showed marked papillary atrophy, a decrease in medullary tissue solute concentrations, and a reduction in AQP2 expression specifically in the inner medulla. Glomerular filtration rate, renal plasma flow and urinary excretion rates of sodium, potassium and chloride did not differ between groups.

The authors concluded that adult rats treated neonatally with enalapril showed a urinary concentrating defect which primarily could be explained by renal papillary atrophy. An impaired ability to generate medullary interstitial hypertonicity and a decrease in inner medullary AQP2 expression seemed to contribute to this defect.

This reviewer's comment: Please note that in both of Guron's studies above (in 1999 and 2005) the rats received enalapril as pups (0-3 days to weaning). The study in 1999 looked at the lasting toxicity in the adults although the rats received enalapril only for a short period after birth while

the 2005 paper looked at the toxicity in weanling rats. The results show that receiving enalapril shortly after birth is nephrotoxic at these supra-pharmacological dosages and the kidney lesions and functional defects can still be observed in the adult.

- Angiotensin-Converting Enzyme Inhibition Modulates Mitogen-Activated Protein Kinase Family Expressions in the Neonatal Rat Kidney. Choi BM, Yoo KH, Bae IS et al. Ped Res, 2005, 57:115-123.

The aim of this study was to examine the relationship between the mitogen activated protein kinase (MAPK) family and renin-angiotensin system during neonatal renal development. Newborn rat pups were treated with enalapril (30 mg/kg/day i.e. about 60 to 70 times the recommended oral dosage of 0.08 mg/kg in a child in mg/M² equivalent) or normal saline for 7 days. Right kidneys of both groups were selected for immunohistochemical stains of MAPKs and activating transcription factor-2 (ATF-2), and left kidneys were selected for reverse transcriptase-PCR and immunoblot analysis of MAPKs, phospho-MAPKs, and ATF-2. To determine whether apoptosis is involved in the same tubules that highly expressed JNK and p38, authors performed terminal deoxynucleotide transferase-mediated nick-end labeling stain for apoptotic cells and immunohistochemical stains for JNK-2, p38, and ATF-2 expression in the serial sections from the same kidney of the enalapril-treated group.

In the enalapril-treated group, JNK-2, p38, phospho-JNK-2, phospho-p38, and ATF-2 protein expressions were significantly increased, and the immunostaining was strongly detected in the proximal tubular epithelial cells in the cortex, compared with the control group. In particular JNK-2 and p38 expressions were highly expressed and correlated with the location of apoptosis. ERK1/2 and phospho-ERK expressions were not changed by enalapril.

The authors suggested that the expressions of the MAPK family members are modulated by angiotensin-converting enzyme inhibition in the developing kidney. JNK and p38 may be involved in angiotensin II-related intracellular signaling pathways leading to renal apoptosis in the developing kidney.

This reviewer's comment: This is a stress/inflammatory pathway, and a non-specific mechanism of toxicity cannot be ruled out at this lethal dosage. Obvious toxicity was demonstrated by high percentages of deaths (close to 30%) at the dosages used in the studies reviewed herein.

- Spironolactone and Enalapril Differentially Up-Regulate the Expression of VEGF and Heme Oxygenase-1 in the Neonatal Rat Kidney. Yim HE, Kim JH, Yoo KH, et al. Ped Res, 2011, 69:378-383.

The authors investigated the relationship between renin-angiotensin-aldosterone system (RAAS) and hypoxia in the developing kidney. The expression of VEGF and heme oxygenase 1 (HO)-1 related with the oxygen content was analyzed in the enalapril- or spironolactone-treated neonatal rat kidneys. Enalapril (30 mg/kg/day orally i.e. about 60-70 times the recommended pediatric dosages of 0.08 mg/kg/day in mg/M² equivalent) or spironolactone was

administered to newborn rat pups for 7 days. The newborn rats were injected ip with pimonidazole 1 hour before killing. Pimonidazole is a marker of severe tissue hypoxia.

VEGF and HO-1 protein expression was significantly increased using immunoblots and immunohistochemistry in both the enalapril- and spironolactone-treated kidneys, compared to controls ($p < 0.05$). HO-1 mRNA expression was increased in the spironolactone-treated group ($p < 0.05$). The immunostaining of pimonidazole was not different from that of the controls in the enalapril-treated group, whereas it was increased in the spironolactone-treated group.

The authors concluded that the results of this study seemed to indicate that aldosterone blockade or angiotensin II inhibition in the developing rat kidney up-regulated renal VEGF and HO-1 expression regardless of the hypoxic conditions and may differentially modulate VEGF and HO-1 production. Again, the dosage used was an LD30% in other weanling rat studies.

- Antithrombotic effect of captopril and enalapril in young rats. Wodzimierz Buczko et al. J Pharmacol, 2004, 56, 97–104.

Angiotensin converting enzyme inhibitors (ACE-Is) are the main drugs used in the treatment of essential hypertension and congestive heart failure in adults. The goal of this study was to demonstrate the beneficial effect of enalapril on hemostasis in young animals.

Two month old rats with induced thrombosis of the vena cava of the abdomen received enalapril acutely at oral dosages of 3, 10, 30 mg/kg i.e. about 9, 30, and 90 times the oral dosage of 0.08 mg/kg in a child in mg/M² and at 45 times this dose (15 mg/kg/day) for 10 days consecutively.

Venous thrombosis of the vena cava of the abdomen of the rat was induced 240 minutes after the administration of enalapril, whereas it was induced on the 11th day (approximately 12 hours after the last administration) of the compound in the 10-day administration scheme.

Parameters evaluated were prothrombin time (PT), activated partial thromboplastin time (APTT), and euglobulin clot lysis time (ECLT) beginning 2 hours after the induction of the venous thrombosis; systolic blood pressure (SBP) measured by the tail cuff method pre-study, and at 4 hours after a single administration of enalapril, or after 10 days of chronic administration of enalapril. Data were analyzed statistically.

Dose level and frequency-dependent reduction in the thrombus weights were observed in rats receiving enalapril once or chronically. Systolic blood pressure was reduced to a similar extent after acute and chronic administration of the drugs. Enalapril also decreased ECLT only when given chronically whereas PT and APTT were not changed.

The authors concluded that enalapril has antithrombotic effects in young animals and that the activation of the fibrinolytic pathway seems to play an important role in the mechanism of enalapril-mediated antithrombotic action.

This reviewer's comment: Once again the dosages used in the animals are so high compared to the clinical dosages that one can question the applicability of this finding although there is some evidence of antithrombotic activity of ACE-i in the adults (Ridker, 1995).

- Control of growth in neonatal pig hearts. Beinlich CJ, and Morgan HE. Mol Cell Biochem. 1993, 119:3-9.

The pig heart grows at a maximal rate in the first 2–3 days of life due to volume overload imposed on the heart at birth and pulmonary artery hypertension. Rates of ribosome formation and protein synthesis cannot be further accelerated during *in vitro* perfusion with agents that increase cyclic AMP, that bind to α 1-adrenergic receptors or to angiotensin II receptors. Growth of the heart *in vivo* can be restrained by treatment with an angiotensin-converting enzyme inhibitor, enalapril maleate, or an angiotensin receptor antagonist, DuP 753.

Newborn piglets were treated for 2-3 days with enalapril maleate at 5 mg/kg/day which corresponded to about 250 times or 60 times the iv or oral administration of 0.02 or 0.08 mg/kg/day in a pediatric patient in mg/M² equivalent (the article did not say if enalapril was provided iv or orally to the piglets hence the 2 bioequivalence given).

When hearts from enalapril-treated pigs were perfused *in vitro*, rates of protein synthesis and ribosome formation in the LV were lower. These studies suggested that angiotensin II is an important factor accounting for rapid growth of the neonatal heart in response to pressure overload at birth (per authors).

Reviewer's comment: Even in this species, enalapril was tested at a highly supra pharmacodynamic dosage.

- Long-term reduction of renal interstitial hydrostatic pressure after neonatal renin-angiotensin system inhibition in the rat. Nilsson ABM, Adams MA, Matthews SJ et al. Nephrol Dial Transplant, 1998, 13:3065-3073.

Neonatal inhibition of the renin angiotensin system (RAS) causes a decreased urinary concentrating ability, papillary atrophy, and tubular interstitial inflammation in the long term. As a consequence of these morphological changes, the authors surmised “that renal blood flow and renal interstitial hydrostatic pressure (RIHP) may be altered during and shortly after cessation of neonatal angiotensin-converting enzyme (ACE) inhibition, and that tentative changes of these variables would persist long after treatment withdrawal.”

Rats were given daily ip injections of enalapril (10 mg/kg or about 80 times the recommended dosage of 0.02 mg/kg IV in mg/M² equivalent) or saline from days 3 to 23 postpartum, and the relationship between renal perfusion pressure (PP) and RIHP was investigated in 6- and 13-week-old anaesthetized rats.

Neonatal ACE inhibition did not affect baseline RIHP short term, whereas RIHP was reduced at 13 weeks of age versus controls (11.6+/-1.6 vs 18.5+/-1.0 mmHg, p<0.05). Changes in RIHP correlated positively to changes in renal PP, independent of treatment and age (slope averaged 0.11+/-0.03). Ongoing ACE inhibition until 6 weeks of age neither affected baseline RIHP nor altered the reactivity to changes in perfusion pressure. Mild renal histopathological abnormalities were present already 3 weeks after cessation of treatment and were aggravated significantly in the 13-week-old rats, showing a complete loss of the papillary parenchyma.

Authors concluded that the reduced baseline RIHP in adult rats seemed to constitute a functional correlate to the major papillary atrophy. However, RIHP response to changes in renal perfusion pressure was maintained, possibly indicating a compensatory effect of the remaining vasa recta and/or peritubular capillary network. Taken together, lack of neonatal angiotensin II type-1 (AT₁) receptor stimulation induces not only irreversible abnormalities of the renal architecture, but causes alteration of intra-renal haemodynamics, such as a reduced RIHP, which may have implications for the regulation of pressure-natriuresis.

- Angiotensin converting enzyme inhibition in the postnatal rat results in decreased cell proliferation in the renal outer medulla. McCausland JE, Ryan GB, and Alcorn D. Clin Exp Pharmacol Physiol. 1996 Jun-Jul; 23(6-7):552-4.

The effects of ACE inhibition were investigated at a stage of development before the renal outer medulla is fully established. Sprague-Dawley rat pups were given daily ip injections of either enalapril at the dosage of 10 mg/kg/day i.e. about 80 times the recommended dosage of 0.02 mg/kg/day IV in the pediatric patient or saline from Days 3-10 after birth. At Day 11, kidneys were prepared for either electron microscopy or immunocytochemistry analysis. Sections were incubated in proliferating cell nuclear antigen (PCNA) antisera and the avidin-biotin immunoperoxidase method was used to detect an immunoreactive product, indicative of proliferating cells.

At Day 11 there were no statistically significant differences in body and kidney weights between control and treated. Following enalapril treatment, the normal structural arrangement of the outer medulla was disrupted compared with controls i.e. dilated tubules were interspersed among normal tubules among normal tubules with an increased proportion of interstitium. Cell proliferation (PCNA-positive cells) in the medullary rays was reduced in enalapril-treated kidneys compared with control kidneys. Decreased cell proliferation was obvious in both interstitial cells and epithelial tubules. The authors concluded that angiotensin II appears to be essential for normal tubular development and vascular growth in the post natal period in the rat. The dosage used was markedly supra-pharmacodynamic.

- Growth hormone aggravates renal abnormalities induced by neonatal enalapril treatment. Nilsson ABM, Friberg P, Bonquist L, et al. Pediatr Nephrol, 2003, 18:876-886.

The authors explored whether growth hormone (GH) which is the strongest secretagogue for insulin like growth factor I (IGF-1) could reproduce the effect of IGF-1 at reversing the kidney abnormalities following suppression of angiotensin II type-1 receptor stimulation in the neonate rat.

Rats were treated orally from 3 to 13 days of age with the angiotensin-converting enzyme inhibitor enalapril (10 mg/kg/day or about 80 times the recommended pediatric dosage of 0.02 mg/kg/day IV in mg/M² equivalent) and GH (4 mg/kg/day), alone or in combination. Renal gene

expression of IGF-I and IGF-binding proteins (IGFBP) was determined during and after treatment. Renal function and morphology were investigated in the adult.

In contrast to the beneficial effect of IGF-I, GH treatment in combination with enalapril further deteriorated both renal function and morphology as compared with the treatment with enalapril alone, demonstrating reduced glomerular filtration rate, increased tubular dilation and further expansion of the outer medulla. Enalapril decreased medullary expression of IGF-I and increased renal expression of IGFBP-1, changes that were not affected by concomitant GH treatment.

The authors concluded that GH and IGF-I have different roles in the renin-angiotensin system-mediated kidney development in the neonatal period in the rat.

- Angiotensin-converting enzyme inhibition decreases growth factor expression in the neonatal kidney. Yoo KH, Wolstenholme J, and Chevalier RL. *Pediatr Res*, 1997, 42:588-592.

This study was designed to investigate the effects of ACE inhibition in the neonatal rat on the expression of genes known to modulate renal cellular proliferation, cell interactions, and extracellular matrix composition. Newborn rat were treated with enalapril from Day 1 after birth (30 mg/kg/day orally or about 60 times the recommended pediatric dosage of 0.08 mg/kg/day in mg/M² equivalent) or vehicle for 14 days, and kidneys were removed for Northern analysis of mRNA for transforming growth factor- β 1 (TGF- β 1), epidermal growth factor (EGF), clusterin, and renin. The distribution of the proteins for TGF- β 1, EGF, and clusterin was also determined by immunohistochemistry.

Enalapril treatment of rat pups resulted in 40% mortality by Day 14, reduced body and kidney weights, decreased glomerular area, and caused tubular dilatation ($p < 0.05$ versus vehicle group). Enalapril decreased renal TGF- β 1 and EGF mRNA expression, and increased renal clusterin and renin expression ($p < 0.05$). Renal tubular EGF protein expression was decreased, while clusterin protein expression was increased after enalapril treatment.

The authors concluded that ACE inhibition in the developing kidney reduces the renal expression of critical growth factors, which may account for renal growth impairment and that clusterin protein expression was indicative of active and increased apoptosis in the developing kidney in rat pups exposed to enalapril.

Reviewer's comment: This study confirms that enalapril dosages of approximately 30-35 mg/Kg are \geq LD 30%.

Animal models of diseases:

- Perinatal Na⁺ overload programs raised renal proximal Na⁺ transport and enalapril-sensitive alterations of Ang II signaling pathways during adulthood. EV Cabral, LD Vieira-Filho, PA Silva, et al. *PLoS One*, 2012, 7(8): e43791.

In this study, the authors investigated the renal mechanisms underlying perinatal Na⁺ overload-programmed alterations in Na⁺ transporters and the renin/angiotensin system (RAS) and the effects of short-term treatment with enalapril in reprogramming the molecular alterations in kidney.

Male Wistar rats were obtained from dams maintained throughout pregnancy and lactation on a standard diet and drinking water (control) or 0.17 M NaCl (saline group). Enalapril (100 mg/l in drinking water or 4 mg/day i.e. at least 120 times the recommended oral dosage in children 1 month and older of 0.08 mg/kg in mg/M² equivalent) was administered for three weeks after weaning. Animals were observed at 90 day old.

Effects in offspring from dams loaded with salt:

Ninety day old offspring from dams that drank a saline solution presented proximal tubules with increased (Na⁺K⁺) ATPase pump expression and activity. Ouabain-insensitive Na⁺-ATPase pump activity remained unchanged but its response to angiotensin II (Ang II) was lost. PKC, PKA, renal thiobarbituric acid reactive substances (TBARS), macrophage infiltration and collagen deposition markedly increased in the kidney interstitium. In the kidney AT₂ receptor expression decreased, AT₁ expression was unaltered while circulating angiotensin I was significantly lower.

Effects of enalapril treatment in weaned rats:

Treatment with enalapril at weaning reduced the expression and activity of (Na⁺K⁺) ATPase pump, partially recovered the response of ouabain-insensitive Na⁺-ATPase pump to Ang II, and reduced PKC and PKA activities in weaned rats from both salt loaded and control dam groups.

Enalapril treatment also reduced AT₂ receptor expression, and increased TBARS, macrophage infiltration and collagen deposition in the kidney of offspring from both control and salt loaded dams.

The authors concluded that maternal Na⁺ overload induced alterations in renal Na⁺ transporter regulation, as well as severe structural lesions in adult offspring. Enalapril was beneficial predominantly through normalizing Na⁺ pumping activities in adult offspring. However, side effects of its use were detected in adult rats treated after weaning including the down-regulation of PKA, PKC and AT₂ receptor expressions and the increase in TBARS, macrophages and collagen deposition that “could impair renal function in later life.”

This reviewer’s comment: The beneficial effects of this very high dosage of enalapril in this study might have been confounded with non specific toxicity.

- Angiotensin-converting enzyme inhibition aggravates renal interstitial injury resulting from partial unilateral ureteral obstruction in the neonatal rat. Chen CO, Park MH, Forbes MS, et al. Am J Physiol Renal Physiol, 2007, 292: F946–F955.

This study examines the effects of ACE inhibition both during and immediately following the period of post-natal nephrogenesis in the neonatal rat subjected to sham operation or partial unilateral ureteral obstruction (UUO) under general anesthesia within the first 48 hours of life.

Rats in group I received enalapril 30 mg/kg/day orally i.e. about 60 to 75 times the recommended oral dosage of 2 mg/M² in the pediatric patient (or vehicle) daily for the first 10 days, while in group II, the 10 days of treatment began 10 days after surgery. Kidneys were harvested at day 21 and analyzed for apoptosis (TUNEL), interstitial macrophages (ED-1 immunohistochemistry), myofibroblasts (alpha-smooth muscle actin), and collagen (Sirius Red).

Treatment with enalapril increased mortality regardless of timing of administration ($p < 0.05$).

Partial UUO delayed glomerular maturation and increased ipsilateral renal macrophage infiltration, alpha-smooth muscle actin and Sirius Red staining. In group I, enalapril increased myofibroblast accumulation in sham-operated kidneys, but not in obstructed kidneys. In contrast, in group II, enalapril further increased macrophage infiltration, myofibroblast proliferation, and collagen accumulation following partial UUO. The relative abundance of components of the kallikrein-kinin system, measured by Western blot, was not altered by partial UUO in the 14- and 28-day-old rat.

The authors concluded that “in contrast to its salutary effects at later ages, ACE inhibition can worsen injury to the partially obstructed kidney during renal maturation even after the completion of nephrogenesis in weanling rats.”

- A functional immature model of chronic partial ureteral obstruction. Beharrie A, Franc-Guimond J, Rodriguez MM, et al. *Kidney Int.* 2004, 65:1155-1161.

This study was designed to measure the local and systemic pathophysiological mechanisms in an immature model of chronic partial unilateral ureteral obstruction (UUO) after completion of glomerulogenesis. A partial UUO was created by the method of "psoas wrap" in young male weanling rats. Control animals were sham operated. Three groups were divided as follows: sham (N= 15), UUO (N= 18), and UUO + angiotensin-converting enzyme (ACE) (N= 16) inhibitor, enalapril at the dosage of 0.2 mg/kg/day orally i.e. **about 0.6 times** the pediatric dosage of 0.08 mg/kg/day in mg/M² equivalent for 21 days. Renal glomerular and tubular functions were determined by creatinine and uric acid clearances. Diuresis was assessed by urine volume, osmolality, and fractional solute excretions from samples above and below the obstruction. Proteinuria was determined by the urine protein/creatinine ratio (Up/c).

Proteinuria was attenuated in UUO + ACE-treated animals. The hyperuricemia of the immature UUO animals was avoided by an increase in the clearance of uric acid in the UUO + ACE-treated group. Fractional solute excretions suggested a diversion of diuresis to the contralateral unobstructed kidney.

The authors concluded that angiotensin blockade during chronic UUO in young rats afforded protection by attenuating proteinuria, promoting uricosuria, and diverting solute diuresis. “These data suggest a complex interaction of local and systemic mechanisms unique to the maturing kidney.”

4.2 Secondary Pharmacology

4.3 Safety Pharmacology

- An attempt to assess the central action of captopril and enalaprilat.

Czarnecka E1, Strzelec J, and Pietrzak B. Med Sci Monit. 2000, 6:491-497.

The influence of captopril and enalaprilat on the central nervous system in laboratory animals has been studied. The effects of the drugs on duration of ethanol- (4 g/kg ip and thiopental- (70 mg/kg ip) induced sleep, body temperature, spontaneous locomotor activity and analgesic properties (hot plate and tail-flick test) have been investigated in mice. Captopril and enalaprilat at the dosages of 5 and 20 mg/kg ip i.e. about 25 or 100 times the maximum dosage of 20 mg daily IV in an adult in mg/M² equivalent were used in single administration or repeated administration for 10 days. Moreover, pharmacoelectroencephalogram (EEG) profile of captopril and enalaprilat in rabbits was studied.

The authors showed that enalaprilat at a single, or repeated administrations in mice decreased the duration of ethanol and thiopental-induced sleep, decreased body temperature, increased pain threshold, but did not influence the spontaneous locomotor activity.

Furthermore enalaprilat at 1 time the maximum dosage of 20 mg daily IV in adult in a single administration and at 20 times that dose for 5 days produced changes in EEG recording in rabbits.

- Rotarod method in young rats and the antidepressive effect: is the rotarod method capable of evaluating antidepressive effects? Morimoto S, and Kito G. Nihon Yakurigaku Zasshi. 1994, 104:39-49. [Article in Japanese]

The possibilities of applying the rotarod method in young rats for evaluating the antidepressive effect were studied. The results were compared with those obtained by the despair test (forced swimming). The rats used in these tests had not been trained previously.

In the rotarod test, antidepressant drugs such as imipramine (30 mg/kg, p.o.), desipramine (10 mg/kg, p.o.), clorgyline (10 mg/kg, p.o.), mianserin (30 mg/kg, p.o.), trazodone (10 mg/kg, p.o.) and clomipramine (30 mg/kg, p.o.) and an ACE inhibitor, enalapril (30 mg/kg, orally i.e. about 7.5 times the maximum daily oral dosage of 40 mg in the adult in mg/M² equivalent), significantly prolonged the time rats were able to remain on the rotating rod in a dose-dependent manner. Diazepam significantly reduced the duration on the rotating rod. Theophylline, caffeine and fenfluramine did not affect the duration on the rotating rod. In the despair test (forced swimming test), clorgyline, enalapril and caffeine significantly reduced the duration of immobility during the forced swimming in a dose-dependent manner. Imipramine and desipramine significantly reduced the duration of immobility during forced swimming. Trazodone and clomipramine did not affect the duration of immobility. Diazepam significantly prolonged the duration of immobility. A highly significant correlation was noted between the results obtained by the rotarod method and those obtained by the despair test. In the traction test,

theophylline and caffeine significantly prolonged the duration during the traction response. However, other drugs did not affect the duration during the traction response. These results demonstrate that the rotarod method in young rats may be applicable for evaluating the antidepressive effect of a variety of drugs.

NB: the article did not say if enalapril was given once or multiple times in this study.

5 Pharmacokinetics/ADME/Toxicokinetics

NA

5.1 PK/ADME

NA

5.2 Toxicokinetics

NA

(If not included in toxicity studies)

6 General Toxicology

6.1 Single-Dose Toxicity

- Acute renal responses to angiotensin-converting enzyme inhibition in the neonatal pig. Nilson and Friberg. *Pediatr Nephrol*, 2000, 14:1071-1076.

Pharmacological interruption or genetic disruption of the renin-angiotensin system before completion of nephrogenesis produces papillary atrophy and an impaired urinary concentrating ability. "The mechanisms involved have yet to be elucidated, but renal hypoperfusion and subsequent ischemia, particularly to the immature renal medulla, may be hypothesized." The acute intra-renal responses to angiotensin-converting enzyme (ACE) inhibition in the newborn piglet were thus investigated by means of regional blood flow distribution, renal interstitial hydrostatic pressure (RIHP), and medullary oxygen tension (PO₂) in the anesthetized 4- to 5-day-old piglet. Moreover, the calcium antagonist nifedipine and the nitric oxide synthesis inhibitor L-NAME were also given in order to reduce renal blood flow by other means. The drugs were given IV in equipotent pressor doses, mimicking ip injections in neonatal rats.

A single dose of enalaprilat (200 microg/kg i.e. about 10 times the recommended pediatric dosage of 0.02 mg/kg IV in mg/M²) reduced mean arterial pressure (MAP) by 14+/-10% (mean+/-SD, P=0.006) and RIHP by 18+/-18% (P=0.001), whereas total renal blood flow and medullary PO₂ remained unchanged. In contrast, nifedipine (0.5 mg/kg) reduced MAP and RIHP by 39+/-8% and 38+/-14%, respectively, total and regional blood flows by 30%-60%, and

medullary PO₂, by 46+/-29% (P=0.001). Acute administration of L-NAME (15 mg/kg) increased MAP by 27+/-10% (P=0.0005), whereas RIHP and renal blood flow decreased by 20%-50%, resulting in a reduction of the medullary PO₂ by 10+/-12% (P=0.05).

The authors concluded that the renal abnormalities observed after neonatal ACE inhibition are not likely to be caused by renal ischemia and the lack of growth factors might be involved.

This reviewer's comment: Note that the lower dosage equivalent compared to the pediatric situation in this study (x10) compared to Choi's (2005) study (x60-70) reviewed herein might recruit a different mechanism of toxicity. For example a very high dosage relative to the recommended dosage in the pediatric patient upregulated a stress pathway consistent with ischemic kidney after enalapril exposure in pup rats [Yin, 1997] whereas at a lower dosage in this piglet study there was no decrease in O₂ pressure in the kidney and the author hypothesized that enalapril exposure did not result in kidney ischemia but rather in "growth factor deficiency that might be responsible for the pathology observed".

6.2 Repeat-Dose Toxicity

6.2.1 Study title

- Glomerular number and size following chronic angiotensin II blockade in the postnatal rat. McCausland JE, Bertram JF, Ryan GB, et al. *Exp Nephrol*, 1997, 5:201-209.

In this study animals received 10 mg/kg/day enalaprilat ip i.e. about 80 to 100 times the maximum pediatric recommended dosage of 0.02 mg/kg/day IV in mg/M² equivalent from postnatal Day 3 to Day 21. Kidneys were observed at Day 28 at weaning.

According to the authors enalapril treatment did not result in changes in glomerular number or size however abnormalities of tubules and associated vessels were evident throughout the kidney and there was "an increased proportion of interstitium". The structural abnormalities were most prominent in the outer medulla and were consistent with the interruption of descent of the loops of Henle and vasa rectae.

- Furosemide treatment, angiotensin II, and renal growth and development in the rat. Lane PH. *Pediatric Research*, 1995, 37 (6):747-755.

In this study weaned rats received enalapril orally 2.5 mg/day i.e. about 75 times the pediatric oral dosage of 0.08 mg/kg in mg/M² equivalent.

To further study the effects of diuretic treatment on normal renal growth and development, weanling male Munich-Wistar rats received no drug, enalapril, furosemide, or both drugs for 6 weeks; morphometric studies were then performed using standard light and electron microscopic techniques. Plasma renin activity was elevated by furosemide treatment. Cortical tubular growth was stimulated in rats receiving furosemide or both drugs but enalapril did not affect cortical tubular growth when compared with untreated animals. Glomerular volume was increased in furosemide-treated animals, primarily due to an increase in the proportion of mesangial cells, whereas enalapril decreased glomerular volume. Furosemide also increased the filtration surface

area per glomerulus whereas enalapril decreased it. Concurrent enalapril treatment blocked the furosemide-induced changes in filtration surface area as well as attenuating overall glomerular and mesangial growth. Glomerular changes correlated with plasma renin activity. Furosemide stimulated glomerular growth, especially of mesangial cells, probably via stimulation of angiotensin II production. Given the relationship of mesangial cell growth and progressive renal disease, furosemide therapy could thus accelerate glomerulosclerosis. Cortical tubular growth also increased with furosemide but not with enalapril.

This reviewer's comment: This study also confirmed that weanling rats treated with enalapril at high dosages don't develop the toxicity observed in the neonatal kidney in rat pups.

- Postnatal time frame for renal vulnerability to enalapril in rats. Guron G, Marcussen N, Nilsson A, et al. J Am Soc Nephrol. 1999, 10:1550-60.

The aim of the present study was to define the post-natal time frame when the rat kidney is vulnerable to an interruption of the renin-angiotensin system. Male Wistar rats received daily injections of enalaprilat ip at the dosage of 10 mg/kg/day i.e. about 80 to 100 times the maximum pediatric recommended dosage of 0.02 mg/kg/day IV in mg/M² equivalent during different age intervals within 3 to 24 day of age [(D3–9); (D10–16); (D17–24); (D3–13); (D14–24), (D3–24)]. Fluid handling and urinary concentrating ability, renal function under pentobarbital anesthesia, and kidney histology using stereological techniques were evaluated in the adult rats.

Enalapril treatment within 3 to 13 day after birth induced abnormalities in renal function and morphology long-term, whereas treatment initiated at 14 day of age did not. The main histologic alterations were papillary atrophy, and a reduction in the volume of tubular epithelial cells in association with an increase in the proportion of the interstitium, throughout the cortex and outer medulla. Functionally, the predominant defect was the impairment in urinary concentrating ability, which correlated with the degree of papillary atrophy.

For the authors, the vulnerable age interval for the induction of irreversible renal abnormalities by enalapril was the first 13 days after birth in the rat. This postnatal time span coincided with the completion of nephrogenesis and a period of marked tubular growth and differentiation, suggesting a pivotal role for angiotensin II in these processes.

In conclusion, this study showed that the vulnerable age interval for the induction of irreversible renal abnormalities by enalapril in the rat is from embryonic Day 15 to post-natal Day 13 and coincided with the completion of nephrogenesis pre-weaning at Day 21.

- Perturbed medullary tubulogenesis in neonatal rat exposed to renin-angiotensin system inhibition. Lasaitiene D, Chen Y, Guron G, et al. Nephrol Dial Transplant. 2003, 18:2534-2541.

This study aimed to determine the time course for development of tubular structural and inflammatory changes and possible cytokine production in the renal medulla of newborn

rats exposed to an angiotensin-converting enzyme (ACE) inhibitor. Additionally, medullary expression of E-cadherin, a marker for tubular formation, was investigated in ACE-inhibited rats.

Newborn rats were exposed (post-natal Days 0-12) to 10 mg/kg enalapril i.e. 80 to 100 times the maximum pediatric recommended dosage of 0.02 mg/kg/day IV in mg/M² equivalent and killed at days 1, 2, 4, 9 and 13. One kidney was used for morphological evaluation and the other for immunohistochemistry, using antibodies directed against monocytes/macrophages, T cells and E-cadherin on frozen sections. In a separate experiment, rats were treated for 9 days and had their kidneys processed for western immunoblot and immunohistochemistry, where antibodies directed against monocyte chemoattractant protein-1 (MCP-1) and tumor necrosis factor-alpha (TNF-alpha) were used on paraffin sections.

In renal medulla from enalapril-treated rats, volume fractions of tubular lumens and interstitium were increased from postnatal Days 2 and 4, respectively, while that of tubular cells was decreased from 4 days of age. Concomitant loss and/or reduction in E-cadherin expression (from Day 2) was observed in dilated medullary tubules of enalapril-treated rats. Furthermore, in the medulla of enalapril-treated rats, the increased number of ED2⁺ (resident macrophages) cells, followed by the increase in ED1⁺ (monocytes/macrophages) and CD4⁺ T cells, was observed at Days 9 and 13, respectively. This was accompanied by increased medullary expression of TNF-alpha at Day 9.

Authors concluded that neonatal ACE inhibition perturbs medullary tubulogenesis, as indicated by tubular dilatation and a lack of E-cadherin expression in these tubules. Macrophage/monocyte-mediated immune response was considered a secondary event, coincidentally associated with the up-regulation of TNF-alpha.

- Angiotensin-converting enzyme inhibition in piglets induces persistent renal abnormalities. Guron G., Sundelin B., Wickman A. et al. Clin Exp Pharmacol Physiol. 1998, 25:88-91.

The aim of the present study was to investigate whether the pig kidney, which “shows a high resemblance to the human kidney”, is dependent on an intact RAS neonatally for normal renal development, analogous with findings in rats.

Piglets received daily ip injections of either enalapril (10 mg/kg i.e. about 500 times the recommended pediatric dosage of 0.02 mg/kg/day IV in mg/M² equivalent) or vehicle from 2 to 24 days after birth. Urine concentrating capacity, renal functional parameters and renal histology were assessed in 8-week-old pigs.

Urine osmolality after a 20-hour water deprivation period was 673+/-55 and 928+/-50 mOsm/kg (p<0.05) in enalapril- and vehicle-treated pigs, respectively. There were no significant differences between groups in plasma creatinine or urea concentrations. Semi-quantitative analysis of renal histology showed significant interstitial fibrosis and inflammation, tubular atrophy and thickened walls of interlobular arteries in enalapril-treated pigs.

The author concluded that an intact RAS is required for normal renal development in the pig, similar to previous observations made in rodents.

This reviewer's comment: The huge dosage used in this study compared to the pharmacologically recommended dosage in a pediatric patient in mg/M² equivalent obscures the clinical relevance of the findings.

- Angiotensin converting enzyme inhibition decreases cell turnover in the neonatal rat heart. Choi JH, Yoo KH, Cheon HW, et al. *Pediatr Res.* 2002, 52:325-332.

To evaluate the role of endogenous angiotensin in cardiac development, the relationship between angiotensin converting enzyme (ACE) inhibition, cell proliferation, apoptosis, and modulators of apoptosis (bcl-2, bcl-xl, and clusterin) was examined in the developing rat heart. Thirty-five newborn rat pups were treated with enalapril (30 mg/kg/day orally i.e. about 60 times the recommended oral dosage of 2 mg/M² in the pediatric patient) or a vehicle (control group) for 7 days, and hearts were removed for RT-PCR and Western blotting of bcl-2, bcl-xl, and clusterin. An additional 10 rat pups were treated with hydralazine (10 mg/kg/day) or a vehicle, to serve as the hypotensive control. Cell proliferation was determined by proliferating cell nuclear antigen (PCNA) immunostaining, and apoptosis was detected using the TUNEL technique.

Enalapril treatment resulted in 24% mortality, reduced body weight, and decreased heart weight ($p < 0.05$). Enalapril decreased proliferating myocytes by 23%, and reduced proliferating cardiac interstitial cells by 8.1% ($p < 0.05$). Enalapril also decreased myocytes apoptosis by 60%, but the proportion of myocytes undergoing apoptosis was 10-fold less than that of proliferating cells. Cardiac bcl-2 mRNA, clusterin mRNA, bcl-2 protein, and bcl-xl protein content were not changed, but clusterin protein expression was decreased by enalapril treatment. Hydralazine did not alter cardiac cell proliferation or apoptosis.

The authors conclude that ACE inhibition decreases cell turnover in the developing rat heart, which may contribute to cardiac growth impairment. "The loss of myocytes may lead to greater myocyte hypertrophy and myocardial damage later in life."

- Role of bradykinin in the antihypertrophic effects of enalapril in the newborn pig heart. Beinlich CJ, Rissinger CJ, Vitkauskas KJ, et al. *Mol Cell Biochem.* 1996, 163-164:77-83.

According to the authors, rapid growth of the left ventricle of the newborn pig heart can be restrained by treating piglets with the angiotensin converting enzyme inhibitor, enalapril maleate. This reduced rate of growth is reflected *in vitro* by reduced rates of ribosome formation and protein synthesis, and may be due to decreased availability of angiotensin II (AII), a potentially hypertrophic agent; decreased numbers of AII receptors; increased availability of bradykinin, a potentially antihypertrophic agent; or reduced hemodynamic load on the left ventricle. Because enalapril decreases degradation of bradykinin, the role of bradykinin as an inhibitor of cardiac growth in the newborn heart was investigated. Addition of 1×10^{-5} M bradykinin and 1×10^{-6} M enalapril to the perfusate of isolated hearts from 2 day old piglets did not significantly alter heart rate, contents of ATP or creatine phosphate or rates of ribosome formation or protein

synthesis during the 1 hour perfusion. Similarly, exposure of myocytes isolated from the left ventricular free wall of piglets to 5×10^{-6} M bradykinin for 72 h did not alter the rate of [3 H]-phenylalanine incorporation into total protein. The reduced rate of left ventricular growth *in vivo* caused by enalapril administration was not reversed by simultaneous treatment with the specific bradykinin receptor antagonist, HOE 140. HOE 140 alone did not alter ventricular growth as compared to hearts from untreated piglets.

The authors concluded that these results demonstrate that the reduced rate of left ventricular growth *in vivo* and the reduced rate of ribosome formation and protein synthesis in the left ventricle *in vitro* after enalapril treatment of piglets was not the result of an inhibitory effect of bradykinin on cardiac growth.

- Neonatal ACE inhibition in rats interferes with lung development. Lasaitiene D, Chen Y, Nannmark U, et al. Clin Physiol Funct Imaging. 2004, 24:65-68.

The authors tested the hypothesis that ACE inhibition influences the post-natal lung development. Rats were given enalapril at the dosage of 10 mg /kg/day i.e. about 20 times the recommended pediatric oral dosage of 0.08 mg/kg in mg/M² equivalent or about 80 times the maximum recommended dosage of 0.02 mg/kg/day IV in a child in mg/M² equivalent, from 0 to 9 days of age and their lungs were examined at day 4 and 9. Lung structure was evaluated by means of light microscopy, and surface tension of bronchoalveolar lavage fluid was measured by means of a Wilhelmy balance.

The authors concluded that neonatal ACE inhibition lowered the surface tension of bronchoalveolar lavage fluid and caused widening of respiratory airspaces and thinning of alveolar septa. The results suggested that early post-natal ACE inhibition in rats interferes with lung development.

NB: the article did not say if enalapril was given orally or ip.

- Renal cortical accumulation of hyaluronic acid in adult rats exposed neonatally to angiotensin-converting enzyme inhibition. Nilsson ABM, Johnsson C, Friberg P et al. Acta Physiol Scand, 2001, 173:343-350.

The present study aimed at determining if renal cortical hyaluronic acid (HA) in the adult rat is correlated to the abnormal morphology and function in rats treated neonatally with the ACE inhibitor enalapril. In adult control rats (23 weeks old), the cortical HA content was very low [about $5 \mu\text{g}^{-1}$ dry weight (d.w.)] and about 1% of the papillary HA content. In rats treated neonatally with enalapril (at Days 3-13 after birth) at the dosage of 10 mg/kg ip (about 80 times the recommended dosage of 0.02 mg/kg iv in mg/M² equivalent), the cortical HA level was 15 times that in control rats already at 21 days after birth, and it persisted at this level during adulthood (at 23 weeks). At 13 weeks the enalapril-treated animals showed markedly reduced ability (-53%) to concentrate urine during 24-h thirst provocation. At 21 days as well as at 23

weeks the enalapril-treated kidneys displayed morphological changes, such as papillary atrophy, dilation of the tubules and cellular infiltration of the cortical tissue. Histochemical staining confirmed the HA quantification assay and revealed a patchy staining for HA located in the same regions as the infiltrating cells.

The authors concluded that the neonatal treatment with the ACE inhibitor enalapril resulted in renal morphological and functional abnormalities during adulthood in the rat. Cortical HA levels were already seriously elevated at Day 21 coexisting with infiltrating cells.

The authors concluded that “besides the known effects of angiotensin II in development, the accumulation of HA in these kidneys may be involved in the genesis of at least the cortical abnormalities in enalapril-treated animals because of the pro-inflammatory effects and water-binding properties of HA”.

This reviewer’s comment: It cannot be excluded that the supra-pharmacological dosages used in this study relative to the recommended dosage in the pediatric population up-regulated non-specific inflammatory pathways. Hyaluronic acid is involved early in inflammation and wound repair in the kidney [Hirschberg, 2005].

- Renin-angiotensin system in neonatal rats: Induction of a renal abnormality in response to ACE inhibition or angiotensin II antagonism. Friberg P, Sundelin B, Bohman S-O, et al. *Kidney Int*, 1994, 45:485-492.

The present study was designed to determine if inhibition of the renin-angiotensin system early in life produced similar effects in different strains of rats, and focused on characterizing the abnormal fluid balance occurring as a consequence of neonatal treatment with ACE inhibitors or angiotensin II blockers. Three-day-old Wistar Kyoto (WKY), Wistar (WR) and spontaneously hypertensive rats (SHR) were given either saline, enalapril ip at the dosage of 10 mg/kg/day or about 80 times the recommended dosage of 0.02 mg/kg/day in a pediatric patient in mg/M², captopril, losartan and the AT₂ blocker, PD123319, in the same amount of volume for 20 days. Treatment was stopped and rats were examined with regard to renal morphology at 4, 14 and 30 weeks of age. In addition, water consumption, urine volume, urine electrolytes and osmolality were analyzed at 14 weeks of age, that is, 10 weeks off treatment.

Early treatment with the ACE inhibitors, enalapril and captopril, and the AT₁ blocker, losartan, but not the AT₂ blocker, PD 123319, in the SHR and in the normotensive strains WKY and WR produced persistent, irreversible histopathological renal abnormalities in adult life, long after the rats had been taken off treatment. These abnormalities consisted of mainly cortical tubulo-interstitial inflammation, various degrees of papillary atrophy and pelvic dilation. These structural renal abnormalities impaired the urine concentrating ability in the treated animals, as evidenced by a reduced urine osmolality, and caused increases in water consumption and diuresis. Enalapril treatment also reduced rat body weights and body weight gains compared to control.

The authors concluded that an important role for angiotensin II in the developing kidney during the first postnatal weeks or even days exists in the development of normal renal function, and

that “this situation should be seriously considered in clinical situations when any extended ACE inhibitor therapy in newborns is discussed”.

- Tubular mitochondrial alterations in neonatal rats subjected to RAS inhibition. Lasaitiene D, Chen Y, Mildaziene V, et al. Am J Renal Physiol, 2006, 290:F1260-F1269.

Pharmacological interruption of the angiotensin II (ANG II) type 1 receptor signaling during nephrogenesis in rats perturbs renal tubular development. This study aimed to further investigate tubular developmental defects in neonatal rats subjected to ANG II inhibition with enalapril administered ip at the dosage of 10 mg/kg/day or about 80 times the recommended dosage of 0.02 mg/kg/day in a pediatric patient in mg/M². Authors evaluated tubular ultrastructural changes using electron microscopy and estimated activity by spectrophotometry or concentrations of succinate dehydrogenase (SDH), cytochromes a and c, which are components of mitochondrial respiratory chain, on postnatal Days 2 and 9 (PD2 and PD9). Renal expression of sodium-potassium adenosine triphosphatase (Na⁺-K⁺-ATPase) and two reflectors of mitochondrial biogenesis [mitochondrial transcription factor A (TFAM) and translocase of outer mitochondrial membrane 20 (TOM20)] also were studied using Western immunoblotting and immunohistochemistry.

Enalapril disrupted inner mitochondrial membranes of developing cortical and medullary tubular cells on PD2 and PD9. These findings were paralleled by impaired mitochondrial respiratory function, as revealed from the changes in components of the mitochondrial respiratory chain, such as decreased cytochrome c level in the cortex and medulla on PD2 and PD9, decreased cytochrome a level in the cortex and medulla on PD2, and diminished cortical SDH activity on PD2 and PD9. Moreover, tubular expression of the most active energy-consuming pump Na⁺-K⁺-ATPase was decreased by enalapril treatment. Renal expression of TFAM and TOM20 was not altered by neonatal enalapril treatment.

Authors concluded that because nephrogenesis is a highly energy-demanding biological process, with the energy being utilized for renal growth and transport activities, the structural-functional alterations of the mitochondria induced by neonatal enalapril treatment may provide the propensity for the tubular developmental defect.

- Impaired angiogenesis in the enalapril-treated neonatal rat kidney. Yim HE, Yoo KH, Bae ES, et al. Korean J Pediatr, 2016, 59:8-15.

The authors investigated the effect of angiotensin II inhibition on angiogenesis in the developing rat kidney. Newborn rat pups were treated orally with enalapril (30 mg/kg/day or about 60 times the recommended pediatric dosage of 0.08 mg/kg/day in mg/M² equivalent) or vehicle (control) for 7 days after birth. Renal histological changes were checked using hematoxylin and eosin staining. The intra-renal expression of vascular endothelial growth factor (VEGF)-A, VEGF receptor 1 (VEGFR1), VEGFR2, platelet-derived growth factor (PDGF)-B, and PDGF receptor-β with Western blotting and immunohistochemistry staining at postnatal Day 8 were

investigated. Expression of the endothelial cell marker CD31 was examined to determine glomerular and peri-tubular capillary density.

Enalapril-treated rat kidneys showed disrupted tubules and vessels when compared with the control rat kidneys. In the enalapril-treated group, intra-renal VEGF-A protein expression was significantly higher, whereas VEGFR1 protein expression was lower than that in the control group ($p < 0.05$). The expression of VEGFR2, PDGF-B, and PDGF receptor- β was not different between the 2 groups. The increased capillary CD31 expression on the western blots of enalapril-treated rat kidneys indicated that the total endothelial cell protein level was increased, while the cortical capillary density, assessed using CD31 immunohistochemistry staining, was decreased.

The authors concluded that impaired VEGF-VEGFR signaling and altered capillary repair may play a role in the deterioration of the kidney vasculature after blocking of angiotensin II during renal development.

7 Genetic Toxicology

NA

7.1 Study title

Conducting laboratory and location:

Study number(s):

Date of study initiation:

Drug lot/batch number:

GLP compliance: [Yes/No]

QA statement: [Yes/No]

Key Study Findings

Purpose

Methods

Results

7.2 Other Genetic Toxicity Studies

NA

7.2.1 Study title

Conducting laboratory and location:

Study number(s):

Date of study initiation:

Drug lot/batch number:

GLP compliance: [Yes/No]

QA statement: [Yes/No]

Key Study Findings

Purpose

Methods

Results

8 Carcinogenicity

NA

8.1 Study title

Conducting laboratory and location:

Study number(s):

Date of study initiation:

Drug lot/batch number:

GLP compliance: [Yes/No]

QA statement: [Yes/No]

CAC Concurrence: [Yes/No]

Key Study Findings

Purpose

Methods

Results

[Note: - Distinguish neoplastic from non-neoplastic findings.
- Also, include summary of FDA statistical analysis of significant tumor findings vs. sponsor's.]

9 Reproductive and Developmental Toxicology

NA

9.1 Study title

Conducting laboratory and location:

Study number(s):

Date of study initiation:

Drug lot/batch number:

GLP compliance: [Yes/No]

QA statement: [Yes/No]

Key Study Findings

Purpose

Methods

Results

10 Special Toxicology Studies

NA

10.1 Study title

Conducting laboratory and location:

Study number(s):

Date of study initiation:

Drug lot/batch number:

GLP compliance: [Yes/No]

QA statement: [Yes/No]

Key Study Findings

Purpose

Methods

Results

11 Integrated Summary and Safety Evaluation

Enalapril (enalaprilat) given during the period of completion of nephrogenesis in the neonatal period (roughly the first 13 days of birth in a rat pup) at 60 times, or more, the recommended pediatric dosages on a mg/M² basis in pup rats and piglets resulted in irreversible damage to the

kidney structure and function. This damage included tubular malformation, vascular defects and kidney dysfunction leading to permanent kidney failure later in life. The proposed mechanisms included activation of stress and inflammatory pathways, apoptosis and the dysregulation of a variety of growth factors. It is questionable though that the toxicity data in these animal models predicts that in the full term newborn situation because of differences in the timing of the kidney genesis and maturation between animals and humans and the supra-pharmacodynamic dosages used in these preclinical experimental studies. However, since the label for Vasotec indicates that the oral pediatric dosage to treat hypertension can be as high as 0.58 mg/kg/day, a calculated safety margin of 70 at 0.08 mg/kg/day becomes a safety margin of 10 at 0.57 mg/kg/day. Similarly, in hypertensive crisis the IV dosage can be as high as 0.1 mg/kg compared to 0.02 mg/kg/day so that a calculated safety margin of 50 at 0.02 mg/kg/day becomes 10 at 0.1 mg/kg/day. It is also well established that the kidney of the human fetus and the neonate are exquisitely sensitive to toxicity of enalapril and other ACE inhibitors, and use in pregnancy is severely restricted if not contraindicated. To better understand and evaluate the toxicity of enalapril in the neonatal and infancy periods in humans, this reviewer proposes that the guinea pig and the mouse be used since, as in the human, nephrogenesis is completed before birth and renal maturation proceeds rapidly during the first post natal weeks [Derelanko, 2014]. Also dosages of enalapril in the animal models should provide exposures more closely representing those reached in the human fetus and the pediatric population to lessen the risk of expression of off-target pathogenetic mechanisms obscuring any specific enalapril effects on kidney histomorphology and function during the period of completion of nephrogenesis in the human fetus and in certain circumstances in the newborn or infant exposed to enalapril. That said it is noteworthy that the high nephrotoxic dosages in neonatal rats were not nephrotoxic in the adult rat.

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13 Appendix/Attachments

NA

Figure 1 [Figure title]

Table 1 [Table title]

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/s/

MURIEL J SAULNIER
07/21/2016

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