CENTER FOR DRUG EVALUATION AND RESEARCH

APPROVAL PACKAGE FOR:

APPLICATION NUMBER

NDA 21-437

Clinical Pharmacology and Biopharmaceutics Review

Office of Clinical Pharmacology and Biopharmaceutics

New Drug Application Filing and Review Form

General Information About the Submission

	Information		Information
NDA Number	21-437	Brand Name	
OCPB Division (I, II, III)	DIV-1	Generic Name	EPLERENONE
Medical Division	CARDIORENAL	Drug Class	ALDOSTERONE RECEPTOR ANTAGONIST
OCPB Reviewer	GABRIEL ROBBIE	Indication(s)	HYPERTENSION
OCPB Team Leader	PATRICK MARROUM	Dosage Form	25 mg, 50 mg, 100 mg TABLETS
		Dosing Regimen	50 mg QD for 4 weeks titrated up to 200 mg QD
Date of Submission	NOVEMBER 29, 2001	Route of Administration	ORAL
Estimated Due Date of OCPB Review	June 30, 2002	Sponsor	SEARLE
PDUFA Due Date	SEPTEMBER 2002	Priority Classification	S
Division Due Date	September 2002		

Clin. Pharm. and Biopharm. Information

	"X" if included at filing	Number of studies submitted	Number of studies reviewed	Critical Comments If any
STUDY TYPE				
Table of Contents present and sufficient to locate reports, tables, data, etc.	X			
Tabular Listing of All Human Studies	X			
HPK Summary	X			
Labeling	X			
Reference Bioanalytical and Analytical Methods	X			
I. Clinical Pharmacology				
Mass balance:	X	1		
Isozyme characterization:	X	5		
Blood/plasma ratio:	X	1		
Plasma protein binding:	X	1		
Pharmacokinetics (e.g., Phase I) -				
Healthy Volunteers-				
single dose:	X	2		
multiple dose:	X	2		
Patients-				
single dose:				
multiple dose:				
Dose proportionality -				
fasting / non-fasting single dose:	X	1		
fasting / non-fasting multiple dose:	X	1		
Drug-drug interaction studies -				
In-vivo effects on primary drug:	X	13		
In-vivo effects of primary drug:	X	13		
In-vitro:	X	1		
Subpopulation studies -				
ethnicity:	X	1		
gender:	X	1		
pediatrics:	X	1		
geriatrics:	X	1		

renal impairment:	X	1		
hepatic impairment:	X	1		
PD:				
Phase 2:	X	1		
Phase 3:				
PK/PD:				
Phase 1 and/or 2, proof of concept:	X	1		
Phase 3 clinical trial:				
Population Analyses -				
Data rich:				
Data sparse:	X	1		
II. Biopharmaceutics				
Absolute bioavailability:	X	1		
Relative bioavailability -				
solution as reference:				
alternate formulation as reference:	X	1		
Bioequivalence studies -				
traditional design; single / multi dose:	X	3		
replicate design; single / multi dose:				
Food-drug interaction studies:	X	2		
Dissolution:				
(IVIVC):				
Bio-wavier request based on BCS				
BCS class				
III. Other CPB Studies				
Genotype/phenotype studies:				
Chronopharmacokinetics				
Pediatric development plan				
Literature References				
Total Number of Studies				

Filability and QBR comments

	"X" if yes	*Comments*
Application filable ?	X	
Comments sent to firm ?		
QBR questions (key issues to be considered)		
Other comments or information not included above		
Primary reviewer Signature and Date		
Secondary reviewer Signature and Date		

CC: NDA 21-437, HFD-850(Lee), HFD-860(Marroum, Mehta, Sahajwalla, Robbie), Biopharm (CDER)

CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS REVIEW

NDA: 21-437 **SUBMISSION DATES:** Original NDA: 11/28/01

IND: — [N(IM)051-3/14/99; N(IM,PI)161-6/13/00; N(IM,IB)277-10/5/01; N(IM,IT)222-1/23/01, N(IT)265-8/15/01, N(IM)095-12/8/99, N(YY)290-1/24/02; N(IM)255-7/3/01; N(IM)144-5/9/00]

TYPE: 1-S

BRAND NAME: Trade Mark Not Approved Yet

GENERIC NAME: Eplerenone

DOSAGE STRENGTH: 25 mg, 50 mg and 100 mg tablets

SPONSOR: Pharmacia

DIVISION OF PHARMACEUTICAL EVALUATION: I

PRIMARY REVIEWER: Gabriel J. Robbie, Ph.D.

TEAM LEADER: Patrick J. Marroum, Ph.D.

TABLE OF CONTENTS

PAGE

RECOMMENDATION 2

EXECUTIVE SUMMARY 3

QUESTION BASED REVIEW 6

Labeling Recommendations 15

Appendix I: Proposed package Insert 17

APPENDIX II: REVIEW OF INDIVIDUAL STUDIES 29

IN VITRO STUDIES

PLASMA PROTEIN BINDING OF [^{14}C]EPLERENONE (SC-66110) AND [^{14}C]SC-70303 IN CD1 MICE, P53 KNOCKOUT MICE, P53 WILDTYPE MICE, RATS, RABBITS AND HUMANS 30

PLASMA PROTEIN BINDING OF EPLERENONE (SC-66110) IN THE RAT, DOG AND MAN 33

BINDING OF [^{14}C]SC-66110 (EPLERENONE) AND [^{14}C]SC-70303 TO HUMAN SERUM ALBUMIN AND ALPHA 1-ACID GLYCOPROTEIN 36

BINDING OF [^{14}C]SC-66110 (EPLERENONE) AND [^{14}C]SC-70303 TO HUMAN SERUM ALBUMIN AND ALPHA 1-ACID GLYCOPROTEIN 39

INTERACTION OF SC-66110 (EPLERENONE) TRANSPORT THROUGH CACO-2 CELL MONOLAYER WITH P-GLYCOPROTEIN SUBSTRATES AND ANTIBODY 39

INHIBITION OF P-GLYCOPROTEIN BY EPLERENONE IN CACO-2 CELLS AND CACO-2 CELLS TRANSFECTED WITH PGY-1 CDNA 43

IN VITRO DRUG-DRUG INTERACTION STUDIES WITH SC-66110 (EPLERENONE) AND CHLORZOXAZONE 50

IN VITRO DRUG-DRUG INTERACTION STUDIES WITH SC-66110 (EPLERENONE) AND DICLOFENAC 53

IN VITRO DRUG-DRUG INTERACTION STUDIES WITH SC-66110 (EPLERENONE) AND 56

METHYLPHENIDATE

DETERMINATION OF POTENTIAL FOR PHARMACOKINETIC INTERACTIONS BETWEEN SC-66110 (EPLERENONE) AND LOSARTAN 60

INHIBITION OF CYTOCHROME P4501A2, CYTOCHROME P4502C9, CYTOCHROME P4502D6, CYTOCHROME P4502C19 AND CYTOCHROME P4503A4 CATALYTIC ACTIVITIES BY EPLERENONE 65

THE POTENTIAL OF SC-66110 (EPLERENONE) TO INDUCE CYTOCHROME P450 ENZYMES IN FRESHLY ISOLATED HUMAN HEPATOCYTES 70

IN VITRO DRUG-DRUG INTERACTION STUDIES WITH SC-66110 (EPLERENONE) AND AMIODARONE 73

IN VITRO DRUG-DRUG INTERACTION STUDIES WITH SC-66110 (EPLERENONE) AND DEXAMETHASONE 76

IN VITRO DRUG-DRUG INTERACTION STUDIES WITH EPLERENONE AND MEPHOBARBITAL 79

IN VITRO DRUG-DRUG INTERACTION STUDIES WITH SC-66110 (EPLERENONE) AND PHENYTOIN 83

IN VITRO DRUG-DRUG INTERACTION STUDIES WITH SC-66110 (EPLERENONE) AND PHENACETIN 86

IN VITRO DRUG-DRUG INTERACTION STUDIES WITH SC-66110 (EPLERENONE) AND DEXTROMETHORPHAN 89

IN VITRO DRUG-DRUG INTERACTION STUDIES WITH SC-66110 (EPLERENONE) AND METOPROLOL 92

IN VITRO DRUG-DRUG INTERACTION STUDIES WITH SC-66110 (EPLERENONE) AND TOLBUTAMIDE 95

IN VITRO DRUG-DRUG INTERACTION STUDIES WITH SC-66110 (EPLERENONE) AND EITHER KETOCONAZOLE OR FLUCONAZOLE 98

SC-66110 (EPLERENONE) INTERACTION STUDIES IN HUMAN LIVER MICROSOMES 101

PHARMACOKINETICS OF EPLERENONE

DOUBLE-BLIND, PLACEBO-CONTROLLED, SINGLE DOSE SAFETY AND PHARMACOKINETIC STUDY OF ORAL SC-66110 (EPLERENONE) IN HEALTHY MALE SUBJECTS 117

EPLERENONE MULTIPLE ORAL DOSE TOLERABILITY AND PHARMACOKINETIC STUDY 125

PHARMACOKINETIC STUDY OF A SINGLE ORAL DOSE OF [^{14}C]SC-66110 (EPLERENONE) 136

ISOLATION AND IDENTIFICATION OF [^{14}C]EPLERENONE METABOLITES IN HUMANS 141

APPEARS THIS WAY
ON ORIGINAL

EPLERENONE PHARMACOKINETIC ASSESSMENT OF MULTIPLE ORAL DOSES OF 100 MG IN HEALTHY AND UNTREATED MILDLY HYPERTENSIVE JAPANESE VOLUNTEERS 144

PHARMACOKINETICS IN SPECIAL POPULATIONS

THE STUDY EFFECT OF AGE AND GENDER ON THE PHARMACOKINETIC PROFILE OF EPLERENONE IN HEALTHY SUBJECTS 152

EPLERENONE SINGLE AND MULTIPLE DOSE PHARMACOKINETIC EVALUATION IN SUBJECTS WITH AND WITHOUT HEPATIC IMPAIRMENT 164

EVALUATION OF THE SINGLE AND MULTIPLE-DOSE PHARMACOKINETICS OF EPLERENONE IN SUBJECTS WITH AND WITHOUT RENAL IMPAIRMENT 170

THE EFFECT OF RACE ON THE PHARMACOKINETIC PROFILE OF EPLERENONE IN HEALTHY, ELDERLY SUBJECTS 189

POPULATION PHARMACOKINETICS OF EPLERENONE IN PEDIATRIC AND ADULT HYPERTENSIVE PATIENTS 197

PHARMACOKINETIC AND PHARMACODYNAMIC ANALYSIS PERFORMED BY CLINICAL PHARMACOLOGY REVIEWER 205

CHARACTERIZATION OF EPLERENONE PHARMACOKINETICS IN PEDIATRIC HYPERTENSIVE PATIENTS 212

IN VIVO DRUG INTERACTIONS

AN OPEN-LABEL, RANDOMIZED, MULTIPLE DOSE, THREE-WAY CROSSOVER STUDY TO ASSESS THE EFFECT OF EPLERENONE ON THE STEADY-STATE PHARMACOKINETIC PROFILE OF DIGOXIN IN HEALTHY ADULT SUBJECTS 221

A SINGLE-BLIND ASSESSMENT OF THE PHARMACOKINETICS AND PHARMACODYNAMICS OF WARFARIN IN THE PRESENCE OF STEADY-STATE LEVELS OF EPLERENONE IN HEALTHY ADULT SUBJECTS 227

THE EFFECT OF FLUCONAZOLE AND KETOCONAZOLE ON THE SINGLE DOSE PHARMACOKINETIC PROFILE OF EPLRENONE IN HEALTHY SUBJECTS 233

APPEARS THIS WAY
ON ORIGINAL

ASSESSMENT OF THE EFFECT OF EPLERENONE (SC-66110) ON THE STEADY-STATE PHARMACOKINETIC PROFILE OF VERAPAMIL HCL IN HEALTHY ADULT SUBJECTS 242

ASSESSMENT OF THE EFFECT OF EPLERENONE ON THE STEADY-STATE PHARMACOKINETIC AND PHARMACODYNAMIC PROFILE OF GLYBURIDE IN SUBJECTS WITH TYPE II NON-INSULIN DEPENDENT DIABETES MELLITUS 250

AN OPEN-LABEL, RANDOMIZED, MULTIPLE DOSE, THREE-WAY CROSSOVER STUDY TO ASSESS THE EFFECT OF EPLERENONE ON THE STEADY-STATE PHARMACOKINETIC PROFILE OF SIMVASTATIN IN HEALTHY ADULT SUBJECTS 257

EFFECT OF MULTIPLE DOSES OF EPLERENONE ON THE SINGLE DOSE PHARMACOKINETIC PROFILE OF MIDAZOLAM IN HEALTHY SUBJECTS 263

THE EFFECT OF EPLERENONE ON THE PHARMACOKINETIC PROFILE OF CISAPRIDE (PROPULSID) IN HEALTHY SUBJECTS 267

THE EFFECT OF EPLERENONE ON THE PHARMACOKINETIC PROFILE OF CYCLOSPORINE IN HEALTHY ADULT SUBJECTS 275

A SINGLE BLIND, RANDOMIZED, MULTIPLE DOSE, THREE-WAY CROSSOVER STUDY TO ASSESS THE EFFECT OF EPLERENONE COADMINISTRATION ON THE STEADY-STATE PHARMACOKINETIC PROFILE OF SAQUINAVIR IN HEALTHY ADULT SUBJECTS 280

THE EVALUATION OF THE EFFECT OF EPLERENONE ON THE STEADY-STATE PHARMACOKINETIC PROFILE OF ERYTHROMYCIN IN HEALTHY ADULT SUBJECTS 286

THE EFFECT OF EPLERENONE ON THE PHARMACOKINETIC PROFILE OF ORAL CONTRACEPTIVES IN HEALTHY FEMALE SUBJECTS 293

THE EFFECT OF ST. JOHN'S WORT ON THE PHARMACOKINETIC PROFILE OF EPLERENONE IN HEALTHY ADULT SUBJECTS 299

BIOEQUIVALENCE AND BIOPHARMACEUTICS

AN OPEN-LABEL, RANDOMIZED, SINGLE DOSE, TWO PERIOD CROSSOVER STUDY OF THE EFFECT OF FOOD ON THE PHARMACOKINETIC PROFILE OF ORAL EPLERENONE IN HEALTHY MALE SUBJECTS 305

THE STUDY OF BIOEQUIVALENCY OF TABLETS AND CAPSULES OF EPLERENONE IN HEALTHY SUBJECTS 309

THE EFFECT OF FOOD, ANTACID AND GRAPEFRUIT JUICE ON THE PHARMACOKINETIC PROFILE OF EPLERENONE IN HEALTHY SUBJECTS HEALTHY FEMALE SUBJECTS 314

THE BIOEQUIVALENCY STUDY OF DIFFERENT PARTICLE SIZE TABLETS OF EPLERENONE IN HEALTHY SUBJECTS 319

WAIVER FOR A BIOEQUIVALENCE STUDY COMPARING CLINICAL AND COMMERCIAL (TO BE MARKETED) FORMULATIONS 323

DISSOLUTION METHOD DEVELOPMENT 327

PHARMACOKINETIC-PHARMACODYNAMIC RELATIONSHIP BY REVIEWER 333

APPEARS THIS WAY
ON ORIGINAL

RECOMMENDATION

The Office of Clinical Pharmacology and Biopharmaceutics has reviewed NDA 21-437 and finds the clinical pharmacology and biopharmaceutics section acceptable provided labeling comments #1 - 4 are adequately addressed. Based on the similarity of the dissolution profiles of the 25 mg, 50 mg and 100 mg clinical and commercial tablets of eplerenone, an in vivo bioequivalence study waiver is granted. The Office of Clinical Pharmacology and Biopharmaceutics finds the sponsor proposed dissolution method of USP Apparatus II (paddles) at 50 rpm and 1000 ml of 0.1 N HCl dissolution media and dissolution specification of not less than [illegible]% (Q) in 30 minutes acceptable.

COMMENTS:

1. Eplerenone blood pressure lowering effect was not different when administered BID or QD. The maximum predicted reduction in trough sitting diastolic blood pressure and sitting systolic blood pressure is about 6-8% of baseline.
2. The hyperforin content in marketed St. John's Wort products vary over 16-fold. In light of this variation a higher induction in CYP 3A4 activity, resulting in significant reduction in eplerenone concentrations, can be expected. Therefore, concomitant administration of St. John's Wort should be avoided in eplerenone patients.
3. The sponsor's proposal to reduce the dose to 50 mg QD with moderate inhibitors of metabolism (erythromycin, verapamil, fluconazole, saquinavir) and reduction to 25 mg QD with potent inhibitors of CYP 3A4 (ketoconazole) is acceptable.
4. The pediatric pharmacokinetic study was conducted in patients between 4 years and 14 years of age only.
   a. Extrapolation of eplerenone pharmacokinetics to pediatric patients less than 4 years cannot be made.
   b. The pharmacodynamics of eplerenone in pediatric patients is not known.
   c. Eplerenone dose was administered with applesauce in subjects <4 years of age. The relative bioavailability of eplerenone when administered with applesauce is not known. If the sponsor proposes to administer eplerenone with applesauce in pediatric patients, the sponsor will have to conduct a study to investigate the effect of applesauce on the bioavailability of eplerenone.

OCPB briefing held on August 20, 2001. Attendees were, Thomas Marciniak, Peter Lee, Sheiw-Mei Huang, John Hunt, Jurgen Venitz, Arzu Selen and Patrick Marroum.

/S/

Gabriel J. Robbie, Ph.D.
Division of Pharmaceutical Evaluation I

FT: Initialed by Patrick J. Marroum, Ph.D.________

cc list: HFD-110 (Karkowsky,Marciniak): NDA 21-437; HFD-860: (Robbie, Marroum, Mehta); CDER Central Document Room

EXECUTIVE SUMMARY

Pharmacia is seeking approval of Eplerenone, an aldosterone receptor antagonist, for the treatment of hypertension. The recommended starting dose is 50 mg once daily increased to 100 mg once daily, which may be increased to 200 mg once daily.

Eplerenone is a low-solubility drug whose absolute bioavailability is not known because of lack of an intravenous formulation. At therapeutic concentrations, eplerenone exhibits moderate plasma protein binding, about 50% following 100 mg dose, mainly to alpha 1-acid glycoprotein. In vitro, eplerenone concentrations 20-fold higher than therapeutic concentrations decreased plasma protein binding to 30-35%.

Eplerenone exists in equilibrium with SC-70303, the inactive lactone form of eplerenone, whose concentrations are <5% of eplerenone concentrations in plasma. Eplerenone is extensively metabolized after oral dosing. The mean %dose excreted as total radioactivity in urine and feces were 67% and 32%, respectively, of which <2% and <5%, respectively, were due to unchanged eplerenone. Eplerenone is primarily metabolized to inactive metabolites - 6β-OH (32%), 6β,21-OH (21%) and 21-OH eplerenone(8%) via CYP 3A4.

Following single (10 mg to 100 mg) and multiple dose administration (100 mg to 1000 mg), eplerenone pharmacokinetics increased less than dose-proportionally, oral clearance ranged between 9-18 L/h, mean Tmax and $T_{1/2}$ ranged from 1-2 hr and 4-9 hr, respectively. Accumulation of eplerenone following once-daily dosing at steady-state was not significant. Eplerenone pharmacokinetics can be described by an 1-compartment model with CL/F of 9.8 L/h and V/F of 45 L. Eplerenone was rapidly absorbed following a short mean lag time of about 16 minutes. Body weight did not influence eplerenone CL/F but had a significant impact on V/F.

Coadministration of a high-fat meal or antacid with eplerenone did not affect eplerenone pharmacokinetics significantly. Coadministration with grapefruit juice increased eplerenone concentrations up to 29%, this increase is not expected to be clinically relevant.

Following single and multiple dosing of eplerenone, Cmax and AUC were similar in young and elderly subjects and male and female subjects. In elderly Black subjects, steady-state eplerenone CL/F adjusted for 70-kg body weight was higher by 36% compared to elderly Caucasians. In order to obtain a similar reduction in blood pressure, elderly Black subjects may need a higher eplerenone dose compared to elderly Caucasians.

Since eplerenone is extensively hepatically metabolized; not surprisingly, in moderate hepatic impairment eplerenone AUC increased up to 47%, while SC-70303 (open-ring form) AUC increased up to 115% following single and multiple dosing. Dosing adjustment is not necessary in mild and moderate hepatic impairment. The pharmacokinetics of eplerenone was not studied in severe hepatic impairment.

In severe renal impairment patients eplerenone Cmax and AUC were higher by 25%-38% and 38%-54% higher, respectively, following single and multiple dosing. Eplerenone CL/F in hemodialysis patients increased by 70%, however only 9.6% of administered dose was removed by dialysis indicating that the renal excretion is not the primary pathway for eplerenone metabolism. The 21% reduction in eplerenone CL/F is unexpected since 2% of administered dose is excreted unchanged in urine. The differences in eplerenone pharmacokinetics in renal impairment and dialysis patients do not warrant any dosage adjustment.

Eplerenone clearance in pediatric patients 4 to 14 years of age and adults was similar. The volume of distribution of eplerenone was dependent on both body weight and age. The typical value of V/F in a patient who is 18 years old and weighs 45 kg is 32 L. The value of V/F increases with exponents 0.656 and 0.0289 with age and body weight, respectively. This difference in V/F will have a significant effect on Cmax of eplerenone, with older and heavier patients having a smaller Cmax compared to pediatric patients administered the same dose.

In vitro, eplerenone concentrations up to 100 μM-600 μM did not inhibit the metabolism of chlorzoxazone, diclofenac, methylphenidate, losartan, amiodarone, dexamethasone, mephobarbital, phenytoin, phenacetin, dextromethorphan, metoprolol, tolbutamide, amlodipine, astemizole, cisapride, diazepam, 17α-ethinylestradiol, fluoxetine, lovastatin, methylprednisolone, midazolam, nifedipine, simvastatin, triazolam, verapamil and warfarin. Eplerenone decreased the disappearance of digoxin in vitro.

In vitro metabolism of eplerenone to SC-71597 was inhibited by cyclosporin, erythromycin, saquinavir, ketoconazole, fluconazole and chlorzoxazone.

Eplerenone is substantially metabolized by CYP3A4, this is evident from increased exposure in presence of CYP3A4 inhibitors such as 200 mg BID ketoconazole and 500 mg BID erythromycin, which respectively increased eplerenone AUC and Cmax by 5.5 fold and 1.7 fold, and 1.8 fold and 61%. The sponsor recommends decreasing the dose of eplerenone to 25 mg QD with ketoconazole. Additionally, CYP3A4 substrates such as 240 mg QD verapamil and 1200 mg TID saquinavir increased eplerenone AUC and Cmax by 98% and 36%, and 107% and 40%, respectively. Other CYP3A4 substrates such as 40 mg QD simvastatin, 20 mg QD cisapride, 400 mg QD cyclosporin, 10 mg QD midazolam and oral contraceptive Ortho-Novum 1/35 ® 28-day Regimen did not alter eplerenone pharmacokinetics. Eplerenone is also metabolized by CYP450 2C9, to a lesser extent compared to CYP 3A4, as evidenced by the 2.2 fold and 1.4 fold increase in eplerenone AUC and Cmax caused by fluconazole inhibition of CYP 2C9 metabolism of eplerenone. In the glyburide interaction study, glyburide 5 mg QD increases eplerenone Cmax and AUC by 50% and 90%, respectively, while 10 mg QD glyburide increases eplerenone Cmax and AUC by 40% and 60%, respectively. The sponsor recommends dose reduction to 50 mg QD when coadministered with erythromycin, verapamil, saquinavir and fluconazole.

Coadministration of eplerenone 100 mg QD with digoxin 200 g QD, and warfarin 1 to 15 mg QD did not significantly alter the pharmacokinetics of either compound, nor did coadministration have a clinically important effect on the anticoagulant activity of warfarin.

Pretreatment with 300 mg TID St. John's Wort for 14 days altered single dose pharmacokinetics of eplerenone by reducing mean Cmax and AUC by 19% and 30%, respectively. The effect of CYP3A4/P-gp induction on the multiple dose pharmacokinetics of eplerenone is not known. The hyperforin content in marketed St. John's Wort products vary over 16-fold. In light of this variation a higher induction in CYP 3A4 activity, resulting in significant reduction in eplerenone concentrations, can be expected. Therefore, concomitant administration of St. John's Wort should be avoided in eplerenone patients.

The proposed dissolution method for eplerenone tablets is USP method II (paddle) at a paddle speed of 50 rpm. The proposed medium for dissolution testing is 0.1 N HCl at 37^{0}C with a specification of Q not less than —% in 30 minutes. The proposed dissolution method and specification are acceptable.

Eplerenone in plasma and urine were measured using a validated —— method. The limit of quantification of eplerenone in plasma and urine were — ng/ml and — ng/ml, respectively.

APPEARS THIS WAY
ON ORIGINAL

QUESTION BASED REVIEW

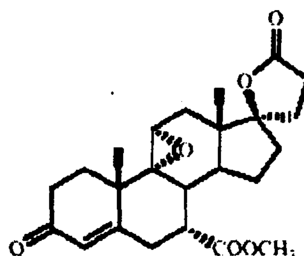
I. INTRODUCTION

A. WHAT ARE THE HIGHLIGHTS OF THE CHEMISTRY, FORMULATION AND PHYSICAL-CHEMICAL PROPERTIES OF THE DRUG AND DRUG PRODUCT?

STRUCTURE

Eplerenone is

Draft



molecular formula: $C_{24}H_{30}O_6$
molecular weight: 414.50

FORMULATION AND MANUFACTURING

Eplerenone tablets do not have a trade name yet. Eplerenone drug substance is odorless, white to off-white crystalline powder. It is to be marketed as 25 mg, 50 mg and 100 mg film coated tablets for oral administration. The compositions of commercial eplerenone tablets are listed in the following table.

	Quantity (mg)/Tablet		
	25 mg	**50 mg**	**100 mg**
Core Tablet			
Eplerenone	25.0	50.0	100.0
Lactose			
Microcrystalline Cellulose			
Croscarmellose Sodium			
Hydroxypropyl Methylcellulose			
Sodium Lauryl Sulfate			
Talc			
Magnesium Stearate			
Core Tablet Weight			
Film Coating			

Film Coated Tablet Weight (mg)	88.825	175.10	350.20

Eplerenone drug substance is manufactured by Pharmacia, Kalamazoo, Michigan. Eplerenone for commercial distribution will be manufactured, packaged, and labeled by Searle Ltd. 99 Jardines Street, Caguas, PR 00725

SOLUBILITY AND PARTITION COEFFICIENT

Eplerenone is slightly soluble in water and is pH independent. The octanol/water partition coefficient of eplerenone at pH 7.0 is 7.1.

B. WHAT IS THE PROPOSED MECHANISM OF ACTION AND THERAPEUTIC INDICATION?

Eplerenone, a steroid nucleus-based molecular mineralocorticoid antagonist, is a selective aldosterone receptor antagonist (SARA) that effectively blocks aldosterone at receptor sites in tissues throughout the body. Elevated levels of aldosterone have been linked to high blood pressure. Eplerenone binds selectively to aldosterone receptors without progestational and antiandrogen side effects.

It is hypothesized that eplerenone could be used with ACE-I and ACE-II to treat patients who are currently on ACE-I and ACE-II treatment but cannot control their aldosterone.

C. WHAT IS THE PROPOSED DOSAGE AND ADMINISTRATION?

The recommended starting dose is 50 mg once daily increased to 100 mg once daily, which may be increased to 200 mg once daily administered orally.

II. CLINICAL PHARMACOLOGY

A. WAS THERE REASONABLE BASIS FOR THE SELECTION OF THE CLINICAL ENDPOINTS, SURROGATE ENDPOINTS OR BIOMARKERS AND WERE THEY MEASURED PROPERLY TO ASSESS EFFICACY AND SAFETY IN CLINICAL PHARMACOLOGY STUDIES?

Yes, efficacy was based on the adjusted mean change in trough from baseline sitting diastolic blood pressure (DBP) and in 24-hour ambulatory blood pressure monitoring (ABPM) DBP and systolic blood pressure.

B. WERE THE CORRECT MOIETIES IDENTIFIED AND PROPERLY MEASURED TO ASSESS CLINICAL PHARMACOLOGY?

Yes, eplerenone and its inactive open-ring form SC-70303 were measured in all studies. The inactive metabolite SC-71597 was measured in plasma and urine in some studies.

ASSAY VALIDATION

The assay method used to quantify eplerenone, SC-70303 and its metabolites was —— This method was sensitive, specific, precise and accurate. The limit of quantification of eplerenone, SC-70303 and SC-71597 in plasma and urine using —— were – ng/ml and – ng/ml, respectively.

C. WHAT ARE THE EXPOSURE-RESPONSE RELATIONSHIPS FOR EFFICACY AND SAFETY?

Eplerenone reduces trough sitting diastolic blood pressure (SiDBP) – the primary end point and trough sitting systolic blood pressure – secondary endpoint. An Emax model best fit the dose-response relationship for both SiDBP and SiSBP over the dose range of 25 mg to 400 mg/day. The reduction in SiDBP and SiSBP with BID dosing was not statistically significantly different from QD dosing. The dose-response relationship between eplerenone dose and SiDBP and SiSBP reduction were shallow. The typical baseline value for SiDBP was 98.7 mmHg which decreased by a maximum of 6.3% of baseline with an ED_{50} of 39 mg. A 100 mg dose administered QD would reduce SiDBP by 4.5% from baseline while a dose of 200 mg QD would decrease SiDBP by 5.3% from baseline. Modeling of SiSBP yielded similar results - the typical baseline value for SiSBP was 151 mmHg that decreases to a maximum of 7.6% of baseline with an ED_{50} of 27 mg.

- ***ARE THE PHARMACOKINETICS OF EPLERENONE DOSE PROPORTIONAL OVER THE PROPOSED DOSING REGIMEN?***

Yes, eplerenone pharmacokinetics is approximately dose proportional over the recommended therapeutic dose range of 25 mg to 200 mg QD. Deviation from dose proportionality is more pronounced over the range of 300 mg to 1000 mg, where Cmax and AUC increase less than dose proportionally.

- ***DO PK PARAMETERS CHANGE WITH TIME?***

No, PK parameters of eplerenone and SC-70303 do not change with time. Eplerenone and SC-70303 steady-state $AUC_{0-\tau}$ were similar to single dose AUC_{0-inf} indicating linear pharmacokinetics.

In healthy volunteers, administration of 100 mg QD results in steady-state mean eplerenone, Cmax, Tmax, CL/F and $T_{1/2}$ of approximately 2000 ng/ml, 12500 ng.h/ml, 1.5-2 hours, 8-9 L/h and 4 hours, respectively.

D. WHAT ARE THE SALIENT FEATURES OF EPLERENONE PHARMACOKINETICS IN HEALTHY VOLUNTEERS?

ABSORPTION

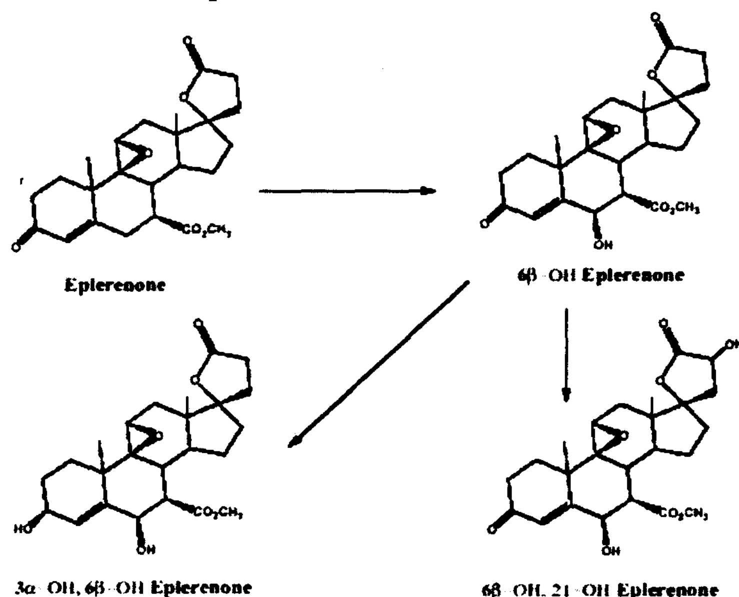
Eplerenone is rapidly absorbed with a mean Tmax of about 1.5 hours to 2 hours. There is a short absorption lag time of 15 minutes. Mean steady-state Cmax and AUC after the 100 mg QD are approximately 2000 ng/ml and 12500 ng.h/ml, respectively. Food and antacid did not affect the absorption of eplerenone.

DISTRIBUTION

Intravenous studies with eplerenone were not conducted because of lack of an intravenous formulation. Therefore, distribution of eplerenone was not characterized. The protein binding of eplerenone is moderate (~50%), binding primarily to alpha 1-acid glycoprotein. Protein binding is saturable and decreases to less than 20% at concentrations 30-fold higher than those achieved with the 100 mg dose.

METABOLISM
Eplerenone is extensively hepatically metabolized primarily by CYP3A4. Eplerenone is primarily metabolized to inactive metabolites - 6β-OH (32%), 6β,21-OH (21%) and 21-OH eplerenone(8%).

Eplerenone Metabolism Scheme is presented below.



EXCRETION
Urinary excretion of unchanged eplerenone accounted for <2%. Elimination of unchanged eplerenone via the feces accounted for <5%. Most of the metabolites of eplerenone are primarily renally excreted.

- ***WHAT IS THE VARIABILITY IN PHARMACOKINETIC PARAMETERS OF EPLERENONE?***

The pharmacokinetics of eplerenone is characterized by moderate variability, about 30%.

E. WHAT DOSAGE REGIMEN ADJUSTMENTS, IF ANY, ARE RECOMMENDED FOR EACH OF THESE GROUPS?

- ***BODY WEIGHT***

Eplerenone clearance is not influenced by body weight. However, eplerenone volume of distribution in adults is related to body weight. The typical value of eplerenone V/F was 37 L in an adult weighing 45 kg which increases with body weight with an exponent of 0.398. For a 70 kg individual the predicted V/F is about 44 L. The difference in Cmax due to differences in volume of distribution is not expected to be clinically signficant.

- ***GENDER***

Following single and multiple oral doses of eplerenone, mean eplerenone plasma pharmacokinetic parameters were not significantly different between female and males except for the amount of eplerenone excreted unchanged in urine: Day 1, Day 7 and Day 14 mean urinary recoveries of eplerenone were higher by 34%, 20% and 4%, respectively, in females compared to males. Mean steady-state eplerenone accumulation (Day 14 to Day 1), was 13% in females while males exhibited a mean accumulation of 6%. Analysis stratified by age and gender indicated that mean accumulation of eplerenone (Day 14 to Day 1) in young males was lower (3%) compared to young female subjects (12%). Similarly, mean accumulation in elderly males was lower (8%) compared to elderly females (15%). The differences in accumulation between gender is not expected to be clinically significant. Therefore, dose adjustment based on gender is not necessary.

- ***RACE***

Following single and multiple dose administration of 100 mg eplerenone, mean Cmax in elderly black subjects was lower by 11% and 19%, respectively, and mean eplerenone AUC were lower by 14% and 26%, respectively, compared to elderly Caucasian subjects. Apparent oral clearance of eplerenone adjusted for 70-kg body weight following single and multiple dosing was higher by 14% and 36%, respectively, in elderly black subjects. The higher CL/F of eplerenone in black subjects is probably attributable to increased metabolism, since a lower, 39% and 40%, mean amount of eplerenone was recovered in the urine following single and multiple dosing, respectively, in elderly black subjects compared to Caucasian subjects. In general, no dosing adjustment is necessary in Blacks. However, in certain subjects a higher dose of eplerenone might be necessary to obtain an equivalent reduction in blood pressure in elderly Blacks compared to Caucasian hypertensive patients.

- ***ELDERLY***

Following a single oral 100 mg dose of eplerenone, mean eplerenone Cmax and AUC in the elderly were higher, 14% and 36%, respectively, compared to young subjects. Mean eplerenone apparent oral clearance adjusted for 70-kg body weight was lower by 27% in the elderly compared to young subjects. A similar difference in pharmacokinetic parameters were observed following multiple dosing too. Mean accumulation at steady-state, Day 14 compared to Day 1 were not very different between young (8%) and elderly (11%) subjects. Dose adjustment based on age is not necessary.

- ***PEDIATRIC PATIENTS***

Eplerenone CL/F of about 10 L/h in hypertensive pediatric patients between 4 and 14 years of age was similar to adults. Body weight did not influence CL/F of eplerenone.

Pediatric patients >4 years dosed with the adult dose of 100 mg would have the same exposure i.e. AUC. This means that patients above 4 years will not need dose correction for body weight to achieve the same AUC as adults. However, the pharmacokinetic-pharmacodynamic relationship of eplerenone in pediatric patients is not known. However, increase in body weight was correlated to increase in the apparent central volume of distribution. The typical value of V/F in a patient who is 18 years old and weighs 45 kg is 32 L. The value of V/F increases with exponents 0.656 and 0.0289 with age and body weight, respectively. Therefore, a 4 year old pediatric patient who weighs about 14 kg is predicted have a V/F of 26 L, while a 35 year old adult patient who weighs 70 kg would have a V/F of 96 L. This difference in V/F will have a significant effect on Cmax of eplerenone, with older and heavier patients having a smaller Cmax compared to pediatric patients administered the same dose.

- ***RENAL INSUFFICIENCY***

Following single and multiple dose administration of 100 mg eplerenone, the greatest effect on eplerenone was observed in severe renal impairment patients where single and multiple dose Cmax of eplerenone was higher by 25% and 38%, respectively, and AUC was higher by 54% and 38%, respectively. Single dose amount of eplerenone excreted in urine in moderate and severe impairment decreased by 54% and 57%, respectively. Multiple dose amount of eplerenone excreted in urine in mild, moderate and severe impairment decreased by 39%, 50% and 37%, respectively. Apparent oral clearance of eplerenone adjusted for 70 kg body weight in mild, moderate and severe renal impairment decreased by 3%, 10% and 21%, respectively, while it increased by 70% in hemodialysis patients. However, only 9.6% of the administered dose was removed by dialysis. The trend of lowered eplerenone CL/F up to 21% with increasing severity of renal impairment is unexpected since 2% of administered eplerenone dose is excreted unchanged in urine indicating that the renal excretion is not the primary pathway for eplerenone metabolism. Comparison of steady-state eplerenone AUC to single dose AUC_{inf} indicated that renal impairment did not affect the linearity of eplerenone pharmacokinetics. Dose adjustment based on renal function is not necessary.

- ***HEPATIC INSUFFICIENCY (HI)***

Eplerenone metabolism was affected by moderate hepatic impairment as evidenced by the higher AUC (42%-47%) in subjects with hepatic impairment compared to normal subjects following both single and multiple oral dosing of 400 mg eplerenone. Mean Cmax of eplerenone was not affected by moderate hepatic impairment. Mean amount of eplerenone excreted unchanged in urine was not affected following a single dose but increased by 87% following multiple dosing in moderate hepatic impairment subjects compared to normal subjects, which indicates a substantial increase in bioavailability due to a decrease in metabolism. SC-70303, open-ring form of eplerenone, concentrations were affected to a larger extent compared to eplerenone. SC-70303 AUC increased by 115% and 101% following single and multiple oral dosing. The amount of SC-70303 excreted in urine in hepatic impairment subjects was higher by 112% and 168% following single and multiple dosing, respectively. Dosing adjustment is not necessary in mild and moderate hepatic impairment. The pharmacokinetics of eplerenone were not studied in severe hepatic impairment patients.

E. WHAT ARE THE EXTRINSIC FACTORS THAT INFLUENCE EXPOSURE OR RESPONSE?

- **FOOD AND ANTACID**

Coadministration of a high-fat meal or antacid did not affect the pharmacokinetics of eplerenone. Eplerenone can be administered without regard to food.

- **GRAPEFRUIT JUICE**

Coadministration of a double strength grapefruit juice increased eplerenone concentrations up to 29%, this increase is not expected to be relevant clinically.

- ***DRUG-DRUG INTERACTIONS***

In-vitro

Eplerenone concentrations up to 100 µM-600 µM did not inhibit the metabolism of chlorzoxazone, diclofenac, methylphenidate, losartan, amiodarone, dexamethasone, mephobarbital, phenytoin, phenacetin, dextromethorphan, metoprolol, tolbutamide, amlodipine, astemizole, cisapride, diazepam, 17α-ethinylestradiol, fluoxetine, lovastatin, methylprednisolone, midazolam, nifedipine, simvastatin, triazolam, verapamil and warfarin. Eplerenone decreased the disappearance of digoxin in vitro. Eplerenone did not inhibit (IC50 =>300 µM) CYP1A2, CYP2C9, CYP2C19 and CYP2D6. Eplerenone inhibited CYP3A4 catalytic activity by approx. 32% at 0.1 µM and approx. 45% at 300 µM. Moderate inhibition of CYP3A4 is expected at therapeutic doses of eplerenone (100mg QD) since mean Cmax of eplerenone following a 100 mg dose is about 1.5 µg/mL, equivalent to 3.6 µM.

In vitro metabolism of eplerenone to SC-71597 was inhibited by cyclosporin, erythromycin, saquinavir, ketoconazole, fluconazole and chlorzoxazone.

Eplerenone was not a substrate of P-Glycoprotein. At expected therapeutic concentrations eplerenone did not induce CYP 3A4 activity.

In-vivo

Eplerenone is substantially metabolized by CYP3A4, this is evident from increased exposure in presence of CYP3A4 inhibitors such as 200 mg BID ketoconazole and 500 mg BID erythromycin, which respectively increased eplerenone AUC and Cmax by 5.5 fold and 1.7 fold, and 1.8 fold and 61%. Additionally, CYP3A4 substrates such as 240 mg QD verapamil and 1200 mg TID saquinavir increased eplerenone AUC and Cmax by 98% and 36%, and 107% and 40%, respectively. However, other CYP3A4 substrates such as 40 mg QD simvastatin, 20 mg QD cisapride, 400 mg QD cyclosporin, 10 mg QD midazolam and oral contraceptive Ortho-Novum 1/35 ® 28-day Regimen did not alter eplerenone pharmacokinetics.

Fluconazole inhibits eplerenone metabolism as evidenced by the 2.2 fold and 1.4 fold increase in eplerenone AUC and Cmax. This is probably a result of CYP 3A4 inhibition by fluconazole.

Coadministration of eplerenone 100 mg QD with digoxin 200 g QD, and warfarin 1 to 15 mg QD did not significantly alter the pharmacokinetics of either compound, nor did coadministration have a clinically important effect on the anticoagulant activity of warfarin.

In the glyburide interaction study eplerenone concentrations when administered alone were not measured. Comparison of eplerenone concentrations from other interaction studies following administration of eplerenone alone indicate that glyburide 5 mg QD increases eplerenone Cmax and AUC by 50% and 90%, respectively, while 10 mg QD glyburide increases eplerenone Cmax and AUC by 40% and 60%, respectively.

Pretreatment with 300 mg TID St. John's Wort for 14 days altered single dose pharmacokinetics of eplerenone by reducing mean Cmax and AUC by 19% and 30%, respectively, probably a result of either induction of CYP 3A4 enzymes by St. John's Wort or increased expression of p-glycoprotein. The decrease in eplerenone exposure might necessitate a higher dose to achieve equivalent reduction in blood pressure. Also, the effect of CYP3A4/P-gp induction on the multiple dose pharmacokinetics of eplerenone is not known.

F. WHAT DOSAGE REGIMEN ADJUSTMENTS, IF ANY, ARE RECOMMENDED?

The hyperforin content in marketed St. John's Wort products vary over 16-fold. In light of this variation a higher induction in CYP 3A4 activity, resulting in significant reduction in eplerenone concentrations, can be expected. Therefore, concomitant administration of St. John's Wort should be avoided in eplerenone patients.

Eplerenone dose should be reduced to 50 mg QD with moderate inhibitors of metabolism (erythromycin, verapamil, fluconazole, saquinavir) and reduced to 25 mg QD with potent inhibitors of CYP 3A4 (ketoconazole) is acceptable.

III. BIOPHARMACEUTICS

A. WAS AN ADEQUATE LINK ESTABLISHED BETWEEN THE CLINICAL AND TO-BE MARKETED FORMULATIONS OF EPLERENONE?

Yes, the sponsor has demonstrated in vivo bioequivalence between the pivotal clinical trial capsules and Phase III tablets. The Phase III clinical trial tablets and the commercial tablets of eplerenone are similar except for shape. The difference in shape between the Phase III tablets and the commercial tablets had no effect on release characteristics of eplerenone drug substance as demonstrated by the similarity in the in vitro dissolution profiles of the 2 tablets.

B. ARE THE SPONSOR PROPOSED DISSOLUTION MEDIUM AND SPECIFICATIONS ACCEPTABLE?

Yes, the Office of Clinical Pharmacology and Biopharmaceutics finds the proposed dissolution method of USP Apparatus II (paddles) at 50 rpm and 1000 ml of 0.1 N HCl dissolution media and dissolution specification of NLT —% (Q) at 30 minutes acceptable.

APPEARS THIS WAY
ON ORIGINAL

APPEARS THIS WAY
ON ORIGINAL

14 pages redacted from this section of the approval package consisted of draft labeling

APPENDIX II

REVIEW OF INDIVIDUAL STUDIES

PLASMA PROTEIN BINDING OF [^{14}C]EPLERENONE (SC-66110) AND [^{14}C]SC-70303 IN CD1 MICE, P53 KNOCKOUT MICE, P53 WILDTYPE MICE, RATS, RABBITS AND HUMANS

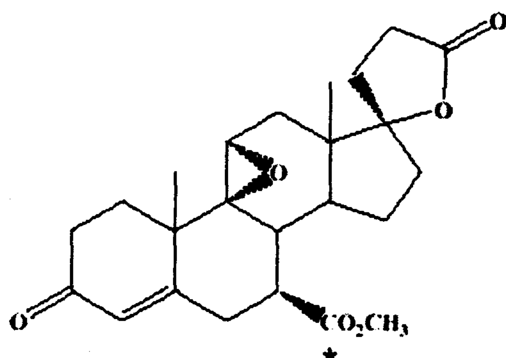
Document #: M3098056

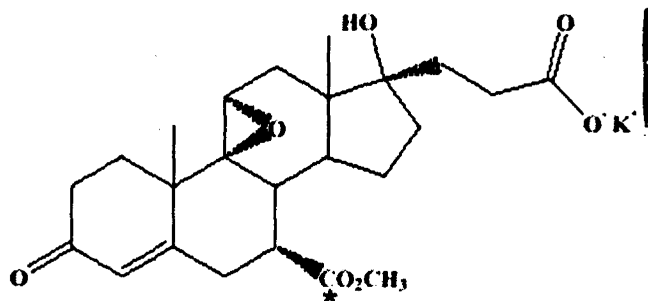
OBJECTIVE:

To determine the binding of [^{14}C]Eplerenone and [^{14}C]SC-70303 to plasma from P53 knockout mouse, P53 wildtype mouse, CD-1 mouse, rat, rabbit and man in the presence of an esterase inhibitor, Esterase Inhibitor C-1.

METHODS:

[^{14}C]Eplerenone and [^{14}C]SC-70303 were labeled at the positions shown in the following figures.





Location of C-14 Label

Location of C-14 Label

Non-specific Binding

Protein-free solutions of [^{14}C]eplerenone and [^{14}C]SC-70303 in the concentration range of 0.0200 to 60.0 mg/mL were filtered through an filtration device.

Plasma Protein Binding

The plasma protein binding of [^{14}C]eplerenone and [^{14}C]SC-70303 were determined by ultrafiltration. [^{14}C]eplerenone and [^{14}C]SC-70303 were added to plasma containing 0.1% Esterase Inhibitor C-1, from human plasma to achieve the desired concentrations. The plasma samples were incubated at 37^{0}C for 60 minutes. Triplicate aliquots of plasma at each concentration were filtered through a filtration device at 2000 x g for

30 min at 25°C. Aliquots of 100-400 mL of ultrafiltrate were collected for analysis of total radioactivity content by Liquid Scintillation Counter.

Calculations

[^{14}C]eplerenone and [^{14}C]SC-70303 bound to plasma at a given concentration was determined using the following equation:

$$Fb = [Ctotal\text{-}Cu]/Ctotal$$

where Cu is the drug concentration recovered in the respective filtrate, and Ctotal is the total drug concentration in plasma. Fb is the fraction of [^{14}C]eplerenone or [^{14}C]SC-70303 bound to plasma. Nonspecific binding of [^{14}C]eplerenone and [^{14}C]SC-70303 to the filtration device was low (<5%), therefore binding results were not corrected for nonspecific binding. The determination of [^{14}C]eplerenone and [^{14}C]SC-70303 peak purity in the incubated samples was performed using

RESULTS:

The mean percentages of protein bound [^{14}C]eplerenone and [^{14}C]SC-70303 over a concentration range of 0.0200-60.0 µg/mL in P53 knockout mice, P53 wildtype mice, CD-1 mice, rats, rabbits and human are given in Table 1.

Table 1. The Mean Percentages of Total Radioactivity Bound to the Plasma of CD-1 Mice, P53 Knockout Mice, P53 Wildtype Mice, Rats, Rabbits and Humans After Incubation with [^{14}C]Eplerenone

Concentration (µg/mL)	P53 Knockout Mouse	P53 Wildtype Mouse	CD-1 Mouse	Rabbit	Rat	Human
	% Bound					
0.0200	28.2	34.8	19.1	20.3	31.2	59.3
0.200	24.3	28.6	19.7	18.8	32.6	57.0
1.00	24.2	27.3	19.3	18.0	32.0	60.9
5.00	22.8	21.3	18.3	19.9	29.5	40.5
20.0	21.5	22.4	8.32	19.5	23.3	39.7
60.0	16.3	19.0	13.6	17.9	20.9	34.3

The binding of [^{14}C]eplerenone to human plasma was moderate (~60%). Plasma protein binding of [^{14}C]eplerenone to human plasma was concentration independent in the 0.0200-5.00 µg/mL range, but appeared to saturate in the 5.00-60.0 µg/mL range. The mean percentages of protein binding were 59, 57, 61, 41, 40 and 34% at concentrations 0.0200, 0.200, 1.00, 5.00, 20.0 and 60.0 µg/mL, respectively.

Table 2. The Mean Percentages of Total Radioactivity Bound to the Plasma of CD-1 Mice, P53 Knockout Mice, P53 Wildtype Mice, Rats, Rabbits and Humans After Incubation with [^{14}C]SC-70303

Concentration (μg/mL)	P53 Knockout Mouse	P53 Wildtype Mouse	CD-1 Mouse	Rabbit	Rat	Human
	% Bound					
0.0200	6.72	13.1	9.25	20.0	18.9	27.8
0.200	6.31	9.52	10.2	17.8	15.1	29.0
1.00	6.00	8.44	9.07	17.6	14.2	24.8
5.00	2.28	10.8	9.39	17.4	17.3	25.9
20.0	1.83	7.26	12.6	16.3	15.2	25.4
60.0	3.41	8.27	9.07	15.7	17.8	32.1

The binding of [^{14}C]SC-70303 to human plasma was similar across the concentration range. The mean percentages of protein binding were 28, 29, 25, 26, 25 and 32% at concentrations 0.0200, 0.200, 1.00, 5.00, 20.0 and 60.0 μg/ml, respectively.

CONCLUSIONS

The binding of [^{14}C]eplerenone to human plasma was moderate (~60%). Plasma protein binding of [^{14}C]eplerenone to human plasma was concentration independent in the 0.02-1.0 μg/ml range, but appeared to saturate in the 5.0-60.0 μg/ml range. The mean percentages of protein binding were 59, 57, 61, 41, 40 and 34% at concentrations 0.02, 0.2, 1, 5, 20 and 60 μg/mL, respectively.

In an in vivo study, after oral administration of [^{14}C]eplerenone to 8 normal healthy male volunteers at a dose of 100 mg the mean percentage of total radioactivity bound to plasma proteins in the 1.5 h post dose sample was 49.4%. The mean concentration of total radioactivity in this sample was 2.39 μg/mL. When [^{14}C]eplerenone was spiked into control plasma at a concentration of 14.5 μg/mL, the percentage of radioactivity bound was 40.4%.

The binding of [^{14}C]SC-70303 to human plasma was similar across the concentration range. The mean percentages of protein binding were between 25% and 32% over a concentration range of 0.02 and 60.0 μg/ml.

APPEARS THIS WAY
ON ORIGINAL

PLASMA PROTEIN BINDING OF EPLERENONE (SC-66110) IN THE RAT, DOG AND MAN

Document #: M3096395

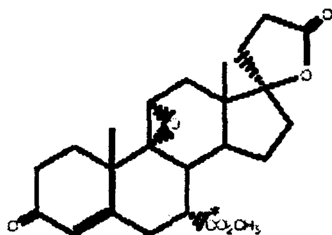
OBJECTIVE:

To determine the plasma protein binding of eplerenone in rat, dog and human plasma.

METHODS:

The plasma protein binding of [^{14}C]eplerenone was determined by an ultrafiltration method as follows: [^{14}C]eplerenone was added to plasma or 0.1 M phosphate buffer (pH 7.4) to achieve the desired concentrations. Triplicate aliquots (0.8 mL) of the plasma sample at each concentration were filtered through a ——— filtration device at approximately 1000 x g for 60 min at approximately 25^{0}C. Approximately 200-400 µL of filtrate was collected from each device. The filters containing the phosphate buffer samples were centrifuged at the same conditions for 15 minutes.

Chemical structure of [^{14}C]eplerenone (SC-66110) indicating the position of the radiolabel is presented below. *=labeled carbon atom.



SC-66110

To determine [^{14}C]eplerenone concentrations in plasma or phosphate buffer, 200 µL aliquots of each sample were added in triplicate to liquid scintillation vials. After centrifugation, a 200 µL aliquot of plasma or phosphate buffer filtrate from each filtration device was also taken for LSC counting.

Eplerenone and SC-70303 %bound was calculated using the following equation.

$$\% \text{ Bound} = 100 - (\frac{[D]_F}{[D]_T} \times 100)$$

where $[D]_F$ is the drug concentration recovered in the respective filtrate, and $[D]_T$ is the total drug concentration in plasma or phosphate buffer. Since nonspecific binding of

[^{14}C]eplerenone to the filtration device was low (3-5%) the percent binding results were not corrected for the nonspecific binding.

RESULTS:

Nonspecific Binding

When phosphate buffer solutions of [^{14}C]eplerenone at different concentrations were filtered through an filtration device, the nonspecific binding (NSB) of [C]eplerenone was low (3-5%) and concentration independent over a concentration range of 0.02 to 60 μg/mL.

Plasma Protein Binding

The mean percentages of plasma protein binding of [^{14}C]eplerenone over a concentration range of 0.02 - 60 μg/mL in rat, dog and human are listed in Table 1.

Table 1. The Mean Percentages of Plasma Protein Bound Eplerenone in Rat, Dog and Human

Concentration (μg/mL)	% Bound		
	Rat	Dog	Human
0.02	25.1 ± 0.7	21.6 ± 0.7	60.6 ± 2.6
0.2	25.2 ± 0.4	15.8 ± 0.4	59.0 ± 3.1
1	18.9 ± 0.1	13.3 ± 0.7	38.2 ± 2.4
5	16.7 ± 0.3	13.4 ± 0.4	33.3 ± 2.1
60	13.1 ± 0.7	14.2 ± 0.9	16.8 ± 1.2

The mean percentages of nonspecific binding of [^{14}C]eplerenone over a concentration range of 0.02 - 60 μg/mL in phosphate buffer is listed in Table 2.

Table 2. Individual Percentages of Nonspecific Bound [^{14}C]Eplerenone in Phosphate Buffer (pH 7.4)

Concentration (μg/mL)	Rep 1	Rep 2	Rep 3	Mean ± SEM
0.02	3.1	3.0	3.5	3.2 ± 0.2
0.2	3.3	3.3	2.1	2.9 ± 0.4
1	2.1	1.9	3.4	2.5 ± 0.5
5	3.0	2.8	3.0	2.9 ± 0.1
60	1.1	0 a	3.1	1.4 ± 0.9

In humans, the plasma protein binding of eplerenone was concentration dependent. The mean percentages of bound eplerenone were 60.6%, 59.0%, 38.2%, 33.3% and 16.8% at concentrations of 0.02, 0.2, 1, 5 and 60 μg/mL, respectively. In a single dose tolerability study of eplerenone in humans, the mean Cmax of eplerenone at following single oral doses of 10, 50, 100, 300 and 1000 mg were 0.191, 0.791, 1.51, 2.98 and 7.31 μg/mL,

respectively. Over the above concentration range of 0.2 to 7 μg/mL, eplerenone is expected to exhibit 30% to 60% protein binding. Since the plasma protein binding over this concentration range is not constant pharmacokinetics of total (bound plus free) eplerenone is expected to be non-linear. However, since plasma protein binding of eplerenone in humans is not extensive (less than 90%), the non-linear pharmacokinetics due to concentration-dependent plasma protein binding is not expected to be of clinical significance.

CONCLUSIONS

The plasma protein binding of [^{14}C]eplerenone is saturable (61% to 17%) over the concentration range of 0.02 to 60 μg/mL and moderate (less than 61% bound).

APPEARS THIS WAY
ON ORIGINAL

APPEARS THIS WAY
ON ORIGINAL

BINDING OF [^{14}C]SC-66110 (EPLERENONE) AND [^{14}C]SC-70303 TO HUMAN SERUM ALBUMIN AND ALPHA 1-ACID GLYCOPROTEIN

Document #: M3198041

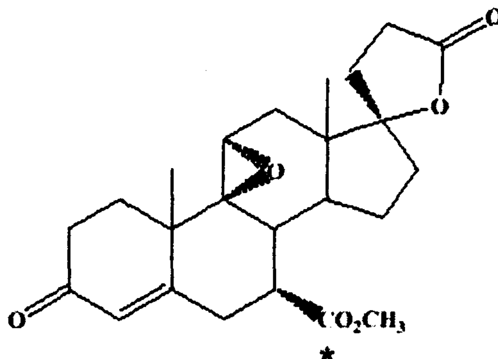
OBJECTIVE:

T o determine the binding of [^{14}C]eplerenone and [^{14}C]SC-70303 in human serum albumin and alpha 1-acid glycoprotein.

METHODS:

Chemical structure of [^{14}C]eplerenone (SC-66110). Asterisk indicates the position of the labeled carbon atom.

The binding of [^{14}C]eplerenone and [^{14}C]SC-70303 was conducted in human serum albumin and alpha 1-acid glycoprotein over a concentration range of 0.0200-60.0 µg/mL using an ultrafiltration method.



* Location of C-14 Label

Non-specific Binding

Solutions of [^{14}C]eplerenone and [^{14}C]SC-70303 over a concentration range of 0.0200 to 60.0 µg/mL were filtered through an . ——— filtration device. Non-specific binding (NSB) of [^{14}C]eplerenone and [^{14}C]SC-70303 was less than 5% and concentration independent over the range of 0.0200 to 60.0 µg/mL.

Specific Protein Binding

The specific protein binding of [^{14}C]eplerenone and [^{14}C]SC-70303 were determined by the following ultrafiltration method: [^{14}C]eplerenone or [^{14}C]SC-70303 were added to protein fortified buffer to achieve the desired concentrations. The specific protein pools were incubated at 37^{0}C for 60 minutes. Triplicate aliquots (0.6 mL) of the protein

fortified sample at each concentration were filtered through a filtration device, at approximately 2000 x g for 30 min at 37°C. Aliquots of 200-400 µL of ultrafiltrate were collected.
Calculations
[^{14}C]eplerenone and [^{14}C]SC-70303 bound to plasma at a given concentration was determined using the following equation:

Fb = [Ctotal-Cu]/Ctotal

where Cu is the drug concentration recovered in the respective filtrate, and Ctotal is the total drug concentration in plasma. Fb is the fraction of [^{14}C]eplerenone or [^{14}C]SC-70303 bound to plasma. Nonspecific binding of [^{14}C]eplerenone and [^{14}C]SC-70303 to the filtration device was low (<5%), therefore binding results were not corrected for nonspecific binding.

RESULTS:

The mean percentages of plasma protein binding of [^{14}C]eplerenone over a concentration range of 0.02 - 60 µg/mL in albumin and alpha 1-acid glycoprotein are listed in Table 1.

Table 1. The Mean Percentages of Protein Bound [^{14}C]Eplerenone in Human Serum Albumin and Alpha 1-Acid Glycoprotein

Concentration (µg/mL)	Albumin	Alpha 1-Acid Glycoprotein
0.0200	11.5	53.7
0.200	5.92	53.0
1.00	6.53	53.3
5.00	7.13	41.2
20.0	7.63	31.1
60.0	8.34	10.5

The mean percentages of plasma protein binding of [^{14}C]SC-70303 over a concentration range of 0.02 - 60 µg/mL in albumin and alpha 1-acid glycoprotein are listed in Table 2.

Table 2. The Mean Percentages of Protein Bound [^{14}C]SC-70303 in Human Serum Albumin and Alpha 1-Acid Glycoprotein

Concentration (µg/mL)	Albumin	Alpha 1-Acid Glycoprotein
0.0200	30.9	2.34
0.200	24.9	0.00
1.00	27.8	0.00
5.00	26.9	0.00
20.0	28.4	0.00
60.0	23.1	0.00

Specific Protein Binding

Mean protein binding of [^{14}C]eplerenone to human serum albumin was almost constant over a concentration range of 0.02 to 60 µg/mL. The mean percentages of protein binding were 11.5, 5.92, 6.53, 7.13, 7.63 and 8.34% at concentrations 0.02, 0.2, 1, 5, 2 and 60 µg/mL, respectively.

Mean protein binding of [^{14}C]eplerenone to alpha 1-acid glycoprotein was concentration independent over a range of 0.02-1.0 µg/ml range. However, above eplerenone concentrations of 1.0 µg/ml protein binding of [^{14}C]eplerenone to alpha 1-acid glycoprotein was saturable. The mean percentages of protein binding were 53.7, 53.0, 53.3, 41.2, 31.1 and 10.5% at concentrations 0.02, 0.2, 1.0, 5.0, 20.0 and 60.0 µg/mL, respectively.

[^{14}C]SC-70303 did not bind to alpha 1-acid glycoprotein except for the 2% binding observed at the lowest concentration of 0.02 µg/mL. Mean protein binding of [^{14}C]SC-70303 to human serum albumin was almost concentration independent over a range of 0.02-60.0 µg/mL. The mean percentages of protein binding were 30.9, 24.9, 27.8, 26.9, 28.4 and 23.1% at concentrations 0.02, 0.2, 1.0, 5.0, 20.0 and 60.0 µg/mL, respectively.

CONCLUSIONS

In vitro, eplerenone predominantly bound to alpha 1-acid glycoprotein. Mean protein binding of [^{14}C]eplerenone to alpha 1-acid glycoprotein was concentration independent, 53-54%, over a range of 0.02-1.0 µg/ml range but above 1.0 µg/ml protein binding of [^{14}C]eplerenone to alpha 1-acid glycoprotein was saturable and declined to 10.5%. Mean protein binding of [^{14}C]eplerenone to human serum albumin was low, <12%, and almost constant over a concentration range of 0.02 to 60 µg/mL.

[^{14}C]SC-70303 protein binding to alpha 1-acid glycoprotein was negligible. Mean protein binding of [^{14}C]SC-70303 to human serum albumin was low, <31%, and almost constant over a concentration range of 0.02-60.0 µg/mL.

APPEARS THIS WAY
ON ORIGINAL

INTERACTION OF SC-66110 (EPLERENONE) TRANSPORT THROUGH CACO-2 CELL MONOLAYER WITH P-GLYCOPROTEIN SUBSTRATES AND ANTIBODY

Document #: M3199193

OBJECTIVE:

To determine whether eplerenone is a substrate for P-gp.

METHODS:

Apical-to-basal (A-to-B) and basal-to-apical (B-to-A) transport of [^{14}C]eplerenone through a Caco-2 cell monolayer system was evaluated at concentrations of 0.05, 0.10, 0.5, 1.0, 5.0, 10 and 50 µM. Caco-2 cells, derived from human colon adenocarcinoma were used. On the 15th day of culture in the , the culture medium was spiked with 11 nM vinblastine sulfate for purposes of P-gp induction (w). Prior to performing the transport studies, the cells were incubated with vinblastine-free medium for approximately 2 hours.

Transport Study

After incubating the with vinblastine-free medium, they were incubated in the transport buffer for additional 15 minutes. Resistance measurements were obtained using a Membranes registering a transepithelial electrical resistance (TEER) of greater than 1100 Ω (350 Ω cm^2) were considered viable.

In the A-to-B transport study, an aliquot (500 µL) of a drug solution was placed in the apical compartment and an aliquot (500 µL) of transport buffer was placed in the basolateral compartment. In the B-to-A transport study, an aliquot (500 µL) of drug solution was placed in the basolateral compartment and an aliquot (500 µL) of transport buffer was placed in the apical compartment. Each concentration was tested in triplicate. The entire 500 µL of sample was collected from compartment B in the A-to-B study and from compartment A in the B-to-A study at 60 and 120 min, and fresh transport buffer was added immediately after each sample collection.

The amount of radioactivity transported was determined by a liquid scintillation counting (LSC) procedure. The percentage of the compound transported (% transported) was calculated for each sample using the following equation:

$$\%\ \text{Transported} = \frac{(\text{DPM in Sample}) \times 100}{\text{Total DPM Added to Donor Compartment}}$$

Where DPM is disintegrations per minute

The net secretion was calculated for each sample using the following equation:

$$\text{Net Secretion} = \frac{\%\ \text{Transported(B-to-A)}}{\%\ \text{Transported(A-to-B)}}$$

RESULTS:

The mean percentages of eplerenone and vinblastine transported over 60 and 120 minutes and net secretion values over the same time periods are given in Tables 1 and 2. The mean values are also shown graphically in Figures 2 and 3. Individual values are given in Tables 1.

Table 1. Mean Transport (%) ± SEM and Net Secretion of Eplerenone and Vinblastine Over 60 Minutes.

Substrate Concentration (µM)	% Transport		Net Secretion
	B to A	A to B	
		Eplerenone	
0.05	6.01 ± 0.16	5.64 ± 0.12	1.07 ± 0.05
0.1	6.03 ± 0.07	5.51 ± 0.06	1.10 ± 0.01
0.5	6.30 ± 0.09	5.81 ± 0.14	1.09 ± 0.04
1	6.30 ± 0.09	5.76 ± 0.01	1.10 ± 0.02
5	5.85 ± 0.22	5.15 ± 0.22	1.14 ± 0.07
10	5.88 ± 0.16	5.71 ± 0.15	1.03 ± 0.01
50	6.32 ± 0.06	5.83 ± 0.17	1.08 ± 0.03
		Vinblastine	
0.05	0.639 ± 0.067	0.148 ± 0.005	4.31 ± 0.31
0.1	0.654 ± 0.066	0.160 ± 0.017	4.10 ± 0.02
0.5	0.661 ± 0.066	0.146 ± 0.007	4.54 ± 0.55
1	0.718 ± 0.071	0.148	4.37
5	0.676 ± 0.039	0.1006 ± 0.0030	6.73 ± 0.37
10	0.756 ± 0.066	0.0989 ± 0.0080	7.66 ± 0.41
50	0.597 ± 0.049	0.0934 ± 0.0200	6.75 ± 0.87

Table 2. Mean Transport (%) ± SEM and Net Secretion of Eplerenone and Vinblastine Over 120 Minutes

Substrate Concentration (µM)	% Transport		Net Secretion
	B to A	A to B	
	Eplerenone		
0.05	11.3 ± 0.3	12.8 ± 0.8	0.889 ± 0.024
0.1	11.3 ± 0.2	11.4 ± 0.1	0.986 ± 0.017
0.5	11.9 ± 0.1	11.4 ± 0.1	1.05 ± 0.02

1	12.0 ± 0.2	11.1 ± 0.0	1.09 ± 0.02
5	11.1 ± 0.3	10.5 ± 0.3	1.06 ± 0.03
10	11.4 ± 0.1	11.3 ± 0.2	1.01 ± 0.01
50	11.8 ± 0.1	11.3 ± 0.4	1.05 ± 0.03
	Vinblastine		
0.05	1.59 ± 0.04	0.309 ± 0.014	5.15 ± 0.11
0.1	1.53 ± 0.00	0.342 ± 0.035	4.55 ± 0.43
0.5	1.50 ± 0.02	0.329 ± 0.009	4.56 ± 0.06
1	1.64 ± 0.10	0.347	4.47
5	1.52 ± 0.08	0.251 ± 0.007	6.04 ± 0.19
10	1.51 ± 0.11	0.263 ± 0.023	5.81 ± 0.47
50	1.22 ± 0.07	0.247 ± 0.033	5.03 ± 0.39

Eplerenone is not a substrate of P-gp. The mean percentages of B-to-A transport for eplerenone were similar to those of A-to-B transport over a concentration range of 0.05 to 50 μM for both 60 and 120 minutes with net secretion values close to 1.0 (from 0.889 to 1.14). Contrary to eplerenone, mean % of B-to-A transport for the prototype P-gp substrate, vinblastine was more than 4-fold higher with net secretion values of 4.17 to 7.91 over 60 minutes and 4.64 to 6.31 over 120 minutes when compared with the values of A-to-B transport.

Mannitol was used as a marker for mono-layer integrity and a negative control for P-gp substrate. Table 3 lists the mean percentages of mannitol transported in eplerenone and vinblastine experiments over 60 and 120 minutes.

Table 3. Mean Transport (%) ± SEM and Net Secretion of Mannitol Over 60 and 120 Minutes.

Time (min)	Experiment	% Transport		Net Secretion
		B to A	A to B	
60	Eplerenone	0.127 ± 0.028	0.130 ± 0.022	0.989 ± 0.139
	Vinblastine	0.120	0.137 ± 0.030	0.870
120	Eplerenone	0.379 ± 0.070	0.376 ± 0.042	0.993 ± 0.088
	Vinblastine	0.407	0.408 ± 0.075	0.896

The mean percentages of B-to-A transport for the negative control, mannitol was low and similar to the values of A-to-B transport in both eplerenone and vinblastine experiments regardless of incubation time indicating maintenance of a viable monolayer during the transport experiments.

CONCLUSIONS

Eplerenone is not a substrate of P-gp. Net secretion of eplerenone across Caco-2 monolayer over a concentration range of 0.05 to 50 μM over 60 and 120 minutes were

close to 1.0, which was much lower than the net secretion values for vinblastine (net secretion 4-6), a prototype P-gp substrate.

APPEARS THIS WAY
ON ORIGINAL

APPEARS THIS WAY
ON ORIGINAL

INHIBITION OF P-GLYCOPROTEIN BY EPLERENONE IN CACO-2 CELLS AND CACO-2 CELLS TRANSFECTED WITH PGY-1 CDNA

Document #: M3001121

OBJECTIVE:

To determine if eplerenone is an inhibitor of P-gp in human carcinoma cell line Caco-2 and transfected cell line Caco-2 with PGY1 CDNA.

METHODS:

Using Caco-2 and Caco-2+PGY1 cell monolayers pretreated with 11 nM vinblastine, apical to basolateral (A-to-B) and basolateral to apical (B-to-A) permeabilities of a low concentration (0.5 µM) of three structurally different and well-known substrates of P-gp, digoxin, vinblastine and doxorubicin, were measured alone and in the presence of two concentrations (50 and 200 µM) of either eplerenone or two P-gp inhibitors, ketoconazole and verapamil, reported in the literature. In addition, A-to-B and B-to-A permeabilities of the substrates were examined with and without P-gp antibody. Mannitol was used as a negative control substrate. Any decreases, relative to control, in the B-to-A/A-to-B permeability ratio (Pr) for each substrate in the presence of inhibitors would indicate a loss of P-gp function and the inhibition of P-gp transport by the inhibitor used. Caco-2 cells, derived from human colon adenocarcinoma and Caco-2 cells transfected with PGY1 cDNA were used.

Prior to performing the transport studies, cell monolayer were rinsed with transport buffer and the monolayer integrity was verified by measuring the transepithelial resistance (TEER) using a . Only wells that displayed a TEER of greater than 300 Ωcm^2 were used in the transport studies. A-to-B and B-to-A permeabilities of a low concentration (0.5 µM) of [^{3}H]digoxin, [^{3}H]vinblastine, [^{14}C]doxorubicin were measured alone and in the presence of two concentrations (50 and 200 µM) of eplerenone as well as the P-gp inhibitors ketoconazole and verapamil. [^{14}C]Mannitol was used as a negative control substrate in the study using the transfected Caco-2 cells. Inhibitors were added to both donor and receiver media. For the A-to-B and B-to-A transport studies, 500 µL of substrate solution (with or without inhibitor) was placed in the appropriate donor chamber and 500 µL of substrate-free buffer (with or without inhibitor) was placed in the receiver chamber. Each sample type was tested in triplicate. To validate the use of [^{3}H]digoxin, [^{3}H]vinblastine and [^{14}C]doxorubicin as P-gp substrates in our Caco-2-system, a low concentration of each was also tested alone and in the presence of 50 µg/mL MRK16 P-gp specific monoclonal in transport buffer.[^{14}C]Mannitol was also tested as a negative control. Control solutions (without antibody) contained 10% phosphate buffered saline (PBS), 0.1% bovine serum albumin and 0.01% sodium azide. Prior to the transport study, the cell monolayers were pre-incubated with 100 µL of 50 µg/mL MRK16 in apical buffer for 30 minutes. During the

transport study, 50 μg/mL MRK16 was also added only to the apical solutions. Each sample type was tested in triplicate. The prepared plates were incubated in a 37 °C water bath oscillating at 80 rpm for the duration of each transport study. Samples were collected after 120 minutes.

Samples were collected from both the apical and basal chambers 120 minutes following the start of the transport study. The samples were analyzed for total ^3H or ^{14}C on a — ——— liquid scintillation analyzer.

Calculations
A-to-B and B-to-A apparent permeabilities (Papp) were calculated using the following equation:

Papp =[(Dr ÷D0)C0V] ÷[AC0T]
Or
Papp =[(Dr ÷D0)V] ÷[AT]*

Where:
Dr = DPM in the receiver chamber after 120 minutes
D0 = DPM in 500 μL of the substrate solution
C0 = Initial substrate concentration in substrate solution (0.5 μM)
V = Volume of receiver chamber (0.5 cm 3)
A = Monolayer surface area (0.31 cm 2)
T = Incubation time (7200 sec)
* This second version of the equation is essentially the same as the first, showing the C0 factors cancelled out.

The permeability ratio (Pr) of a substrate (with or without inhibitor) was calculated by dividing the Papp in the B-to-A direction by the Papp in the A-to-B direction. A ratio of greater than unity indicated a net flux in the B-to-A direction, demonstrating the activity of P-gp. Any decreases toward unity, relative to control, in Pr for each substrate in the presence of inhibitors would indicate the inhibition of P-gp transport by the inhibitor used.

RESULTS:

Table 1 lists **mean** A-to-B Papp,B-to-A Papp and permeability ratio (Pr) values for digoxin, vinblastine and doxorubicin in Caco-2 cell system in the absence and presence of eplerenone, ketoconazole and verapamil.

Table 1. Inhibition of P-gp Transport by Eplerenone, Ketoconazole and Verapamil in Caco-2 Cells

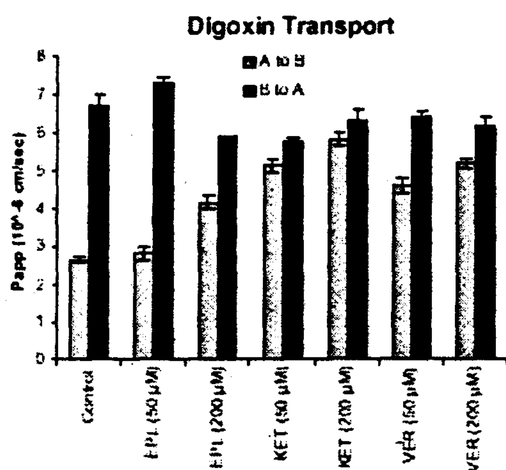
Substrate solution	Papp A-to-B	Papp B-to-A	Pr
1 Digoxin (0.5 μM)	2.64 ± 0.08	6.71 ± 0.31	2.54 ± 0.08
2 ...+Eplerenone (50 μM)	2.81 ± 0.19	7.30 ± 0.14	2.61 ± 0.15

3 ...+Eplerenone (200 μM)	4.15 ± 0.15	5.89 ± 0.01	1.42 ± 0.05
4 ...+Ketoconazole (50 μM)	5.14 ± 0.19	5.73 ± 0.15	1.12 ± 0.01
5 ...+Ketoconazole (200 μM)	5.81 ± 0.18	6.29 ± 0.33	1.08 ± 0.03
6 ...+Verapamil (50 μM)	4.61 ± 0.20	6.37 ± 0.21	1.38 ± 0.04
7 ...+Verapamil (200 μM)	5.20 ± 0.11	6.13 ± 0.28	1.18 ± 0.04
8 Vinblastine (0.5 μM)	0.569 ± 0.014	2.21 ± 0.07	3.90 ± 0.20
9 ...+Eplerenone (50 μM)	0.629 ± 0.049	2.02 ± 0.07	3.24 ± 0.21
10 ...+Eplerenone (200 μM)	0.620 ± 0.049	1.63 ± 0.02	2.67 ± 0.23
11 ...+Ketoconazole (50 μM)	1.00 ± 0.04	1.74 ± 0.06	1.74 ± 0.03
12 ...+Ketoconazole (200 μM)	1.23 ± 0.01	1.55 ± 0.02	1.26 ± 0.02
13 ...+Verapamil (50 μM)	1.15 ± 0.02	1.60 ± 0.01	1.39 ± 0.03
14 ...+Verapamil (200 μM)	1.35 ± 0.11	1.61 ± 0.00	1.21 ± 0.09
15 Doxorubicin (0.5 μM)	0.190 ± 0.015	0.447 ± 0.024	2.36 ± 0.08
16 ...+Eplerenone (50 μM)	0.175 ± 0.024	0.476 ± 0.039	2.77 ± 0.17
17 ...+Eplerenone (200 μM)	0.166 ± 0.007	0.409 ± 0.041	2.47 ± 0.21
18 ...+Ketoconazole (50 μM)	0.367 ± 0.023	0.641 ± 0.026	1.76 ± 0.07
19 ...+Ketoconazole (200 μM)	0.409 ± 0.032	0.558 ± 0.031	1.37 ± 0.05
20 ...+Verapamil (50 μM)	0.309 ± 0.014	0.433 ± 0.021	1.40 ± 0.06
21 ...+Verapamil (200 μM)	0.303 ± 0.035	0.496 ± 0.030	1.68 ± 0.23

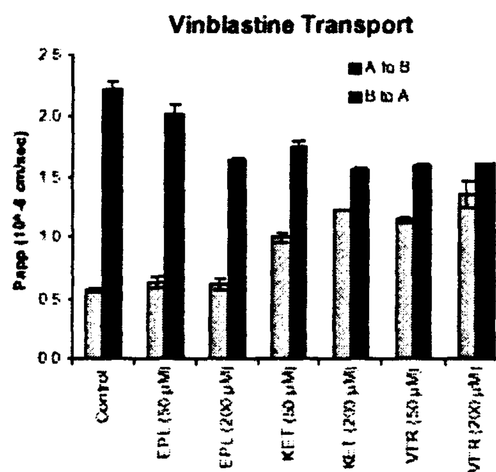
Papp = 10^-6 cm/sec (Mean ± SEM)

Eplerenone 50 μM did not affect A-to-B or B-to-A transport, resulting in a Pr of 2.61, similar to the control value (2.54). However, at the high concentration of eplerenone (200 μM), A-to-B apparent permeability of digoxin increased notably and the Pr was reduced (by 44%) compared with the control value, indicating moderate inhibition of P-gp mediated transport of digoxin at eplerenone concentration of 200 μM. Ketoconazole and verapamil at concentrations of 50 and 200 μM reduced digoxin Pr more than 50%. Thus digoxin transport mediated by P-gp was markedly inhibited by ketoconazole and verapamil at both 50 and 200 μM.

Inhibition of Digoxin Transport by Eplerenone (EPL), Ketoconazole (KET) and Verapamil (VER) in Caco-2 Cells



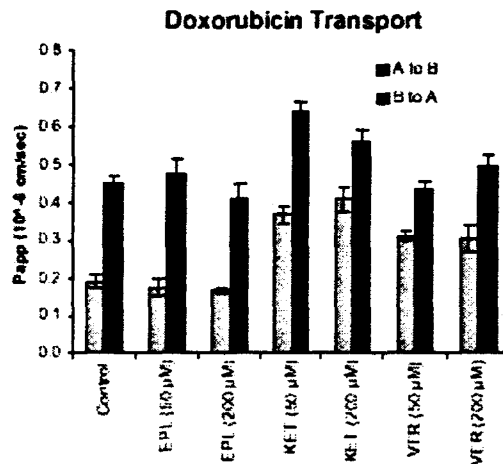
Inhibition of Vinblastine Transport by Eplerenone (EPL), Ketoconazole (KET) and Verapamil (VER) in Caco-2 Cells



Similarly, ketoconazole and verapamil at concentrations of 50 and 200 μM reduced vinblastine Pr more than 50% and 60%, respectively. Thus vinblastine transport mediated by P-gp was markedly inhibited by ketoconazole and verapamil at both 50 and 200 μM.

As seen with digoxin, 50 µM eplerenone did not change the permeability of vinblastine, resulting in a Pr value of 3.24 similar to the control value (3.90). However, 200 µM eplerenone reduced B-to-A permeability by 32% but A-to-B Papp for vinblastine was similar to the control value. Therefore, 200 µM eplerenone moderately inhibited P-gp mediated transport of vinblastine.

Inhibition of Doxorubicin Transport by Eplerenone (EPL), Ketoconazole (KET) and Verapamil (VER) in Caco-2 Cells



Eplerenone 50 µM and 200 µM, did not alter the A-to-B or B-to-A permeability of doxorubicin. However, doxorubicin Pr decreased more than 25% with ketoconazole, and by 40% with verapmail indicating substantial inhibition of transport of doxorubicin mediated by P-gp in Caco-2 cells.

Caco-2+PGY1 Cell Permeability

Table 2 lists mean A-to-B Papp,B-to-A Papp and Pr values for digoxin, vinblastine and doxorubicin obtained using the Caco-2+PGY1 cell system in the absence and presence of eplerenone, ketoconazole and verapamil.

Table 2. Inhibition of P-gp Transport by Eplerenone, Ketoconazole and Verapamil in Caco-2+PGY1 Cells

Substrate solution	Papp A-to-B	Papp B-to-A	Pr
1 Digoxin (0.5 µM)	2.50 ± 0.09	5.38 ± 0.09	2.16 ± 0.09
2 ...+Eplerenone (50 µM)	2.71 ± 0.06	5.63 ± 0.05	2.08 ± 0.04
3 ...+Eplerenone (200 µM)	3.10 ± 0.18	4.54 ± 0.16	1.47 ± 0.04
4 ...+Ketoconazole (50 µM)	3.93 ± 0.08	3.95 ± 0.03	1.01 ± 0.01
5 ...+Ketoconazole (200 µM)	3.90 ± 0.13	3.70 ± 0.10	0.949 ± 0.018
6 ...+Verapamil (50 µM)	3.51 ± 0.11	4.50 ± 0.08	1.28 ± 0.03
7 ...+Verapamil (200 µM)	4.10 ± 0.40	4.31 ± 0.06	1.07 ± 0.08
8 Vinblastine (0.5 µM)	0.900 ± 0.063	2.13 ± 0.12	2.38 ± 0.19

9 ...+Eplerenone (50 μM)	0.760 ± 0.007	2.04 ± 0.06	2.69 ± 0.06
10 ...+Eplerenone (200 μM)	0.766 ± 0.097	1.90 ± 0.06	2.53 ± 0.22
11 ...+Ketoconazole (50 μM)	0.954 ± 0.028	1.18 ± 0.03	1.24 ± 0.04
12 ...+Ketoconazole (200 μM)	0.892 ± 0.008	1.20 ± 0.02	1.35 ± 0.03
13 ...+Verapamil (50 μM)	0.745 ± 0.026	1.48 [a]	2.03 [a]
14 ...+Verapamil (200 μM)	0.888 ± 0.052	1.48 ± 0.05	1.68 ± 0.13
15 Doxorubicin (0.5 μM)	0.396 ± 0.021	0.515 ± 0.009	1.31 ± 0.05
16 ...+Eplerenone (50 μM)	0.366 ± 0.008	0.701 ± 0.118	1.91 ± 0.29
17 ...+Eplerenone (200 μM)	0.379 ± 0.003	0.427 ± 0.005	1.13 ± 0.01
18 ...+Ketoconazole (50 μM)	0.663 ± 0.015	0.676 ± 0.021	1.02 ± 0.01
19 ...+Ketoconazole (200 μM)	0.732 ± 0.016	0.671 ± 0.014	0.918 ± 0.038
20 ...+Verapamil (50 μM)	0.468 ± 0.037	0.653 ± 0.025	1.43 ± 0.18
21 ...+Verapamil (200 μM)	0.611 ± 0.017	0.707 ± 0.011	1.16 ± 0.02
22 Mannitol (0.5 μM)	2.06 ± 0.14	1.70 ± 0.07	0.833 ± 0.046
23 ...+Eplerenone (50 μM)	2.02 ± 0.12	1.83 ± 0.13	0.909 ± 0.026
24 ...+Eplerenone (200 μM)	1.65 ± 0.10	1.77 ± 0.04	1.08 ± 0.07
25 ...+Ketoconazole (50 μM)	1.70 ± 0.17	1.47 ± 0.03	0.881 ± 0.099
26 ...+Ketoconazole (200 μM)	1.77 ± 0.10	1.49 ± 0.10	0.845 ± 0.073
27 ...+Verapamil (50 μM)	1.94 ± 0.13	1.51 ± 0.04	0.785 ± 0.067
28 ...+Verapamil (200 μM)	2.06 ± 0.04	1.79 ± 0.09	0.870 ± 0.051

Papp = 10^-6 cm/sec (Mean ± SEM)

Eplerenone reduced the permeability ratio of digoxin by approximately 30% compared to control value at the high concentration of 200 μM, eplerenone appeared to moderately inhibit P-gp mediated transport of digoxin in the P-gp transfected Caco-2 cell system. Digoxin transport mediated by P-gp was also strongly inhibited by ketoconazole and verapamil in the P-gp transfected cell system. Digoxin Pr was reduced more than 50% with ketoconazole and more than 40% with verapamil at 20 and 50 μM concentrations.

Eplerenone at both 50 and 200 μM concentrations did not affect vinblastine or doxorubicin Pr. The permeability ratio of B-to-A/A-to-B for vinblastine was reduced more than 40% with ketoconazole and more than 25% with verapamil at both 50 and 200 μM. Contrary to digoxin and vinblastine, neither A-to-B nor B-to-A permeability of doxorubicin changed notably with ketoconazole and verapamil at concentrations of both 50 and 200 μM in the Caco-2+PGY1 cell system.

When permeability of mannitol, which is not a substrate of P-gp, was examined in the Caco-2+PGY1 cell system, the permeability did not change with ketoconazole and verapamil regardless of the inhibitor concentrations, resulting in no changes in the Pr. The permeability and permeability ratio of mannitol also did not change with eplerenone.

Immunoinhibition of P-gp Transport

Tables 3 and 4 list the mean permeability of digoxin, vinblastine, doxorubicin and mannitol in the absence and presence of MRK16 anti-P-gp antibody in both Caco-2 and Caco-2+PGY1 cell systems.

Table 3. Inhibition of P-gp Transport by MRK16 P-gp Antibody in Caco-2 Cells

Substrate solution	Papp A-to-B	Papp B-to-A	Pr
1 Digoxin (0.5 μM)	3.48 ± 0.07	8.76 ± 0.07	2.52 ± 0.03
2 ...+MRK16 (50 μg/mL)	5.28 ± 0.04	7.43 ± 0.45	1.41 ± 0.08
3 Vinblastine (0.5 μM)	0.845 ± 0.021	3.64 ± 0.23	4.32 ± 0.38

4 ...+MRK16 (50 μg/mL)	2.61 ± 0.13	3.69 ± 0.17	1.41 ± 0.05
5 Doxorubicin (0.5 μM)	0.588 ± 0.011	0.924 ± 0.024	1.57 ± 0.07
6 ...+MRK16 (50 μg/mL)	0.445 ± 0.018	0.605 ± 0.025	1.36 ± 0.04
7 Mannitol (0.5 μM)	1.28 ± 0.05	1.69 ± 0.10	1.32 ± 0.08
8 ...+MRK16 (50 μg/mL)	1.27 ± 0.07	1.46 ± 0.16	1.15 ± 0.06

Papp = 10^-6 cm/sec (Mean ± SEM)

With MRK16 anti-P-gp antibody, there was a substantial reduction in digoxin and vinblastine Pr in both Caco-2 and Caco-2+PGY1 cell systems. The permeability of doxorubicin was not reduced for either direction (A-to-B or B-to-A) in the presence of the MRK14 P-gp antibody when compared to the control values and there was no substantial changes in the Pr in both Caco-2 and Caco-2+PGY1 cell systems. Similar phenomena were observed with mannitol.

Table 4. Inhibition of P-gp Transport by MRK16 P-gp Antibody in Caco-2+ PGY1 Cells

Substrate solution	Papp A-to-B	Papp B-to-A	Pr
1 Digoxin (0.5 μM)	3.05 ± 0.18	9.37 ± 0.19	3.09 ± 0.14
2 ...+MRK16 (50 μg/mL)	4.02 ± 0.06	6.99 ± 0.06	1.74 ± 0.01
3 Vinblastine (0.5 μM)	0.634 ± 0.072	3.38 ± 0.17	5.42 ± 0.45
4 ...+MRK16 (50 μg/mL)	0.974 ± 0.049	2.79 ± 0.11	2.87 ± 0.04
5 Doxorubicin (0.5 μM)	0.580 ± 0.003	1.02 ± 0.04	1.77 ± 0.08
6 ...+MRK16 (50 μg/mL)	0.387 ± 0.016	0.506 ± 0.011	1.31 ± 0.09
7 Mannitol (0.5 μM)	2.04 ± 0.30	2.67 ± 0.02	1.38 ± 0.23
8 ...+MRK16 (50 μg/mL)	1.43 ± 0.07	1.24 ± 0.08	0.862 ± 0.024

Papp = 10^-6 cm/sec (Mean ± SEM)

The above experiments show that permeability ratios of the P-gp substrates digoxin and vinblastine are markedly reduced by the presence of chemical inhibitors of P-gp such as ketoconazole and verapamil, and also by MRK16 anti-P-gp antibody in both Caco-2 and Caco-2+PGY1 cell systems. The Pr of doxorubicin was also substantially reduced by ketoconazole and verapamil in the Caco-2 cell system. However, in the Caco-2+PGY1 cell system, the permeability ratio of doxorubicin was reduced moderately by ketoconazole at both concentrations and by verapamil only at the high concentration. With MRK16 P-gp antibody, the permeability ratio of doxorubicin did not change in both Caco-2 and Caco-2+PGY1 cell systems. Based on these results, of the three substrate tested, only digoxin and vinblastine were good P-gp substrates to use in screening for potential P-gp inhibitors in the Caco-2 cell systems.

A low concentration of eplerenone (50 μM) did not change the Pr for digoxin and vinblastine in either Caco-2 or Caco-2+PGY1 cell systems. However, at the high concentration, eplerenone reduced the permeability ratios of digoxin and vinblastine to some extent, suggesting that eplerenone is a moderate P-gp inhibitor at high concentrations.

CONCLUSIONS

Eplerenone is not an inhibitor of P-gp transport system at the 50 µM concentration. However at a higher concentration of 200 µM eplerenone was a moderate inhibitor of P-gp transport. Digoxin and vinblastine are good substrates to use in Caco-2 cell systems in the screening of potential P-gp inhibitors, as demonstrated by marked reduction in their permeability ratios by ketoconazole, verapamil and MRP16 anti-P-gp antibody.

APPEARS THIS WAY
ON ORIGINAL

APPEARS THIS WAY
ON ORIGINAL

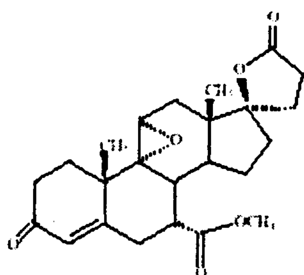
IN VITRO DRUG-DRUG INTERACTION STUDIES WITH SC-66110 (EPLERENONE) AND CHLORZOXAZONE

Study ID: M2100332

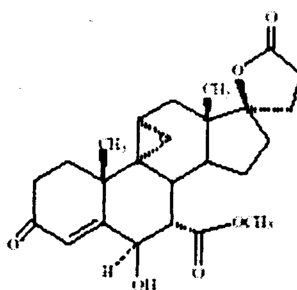
OBJECTIVES:

1. To assess the potential for eplerenone to affect in vitro CYP2E1-mediated formation of 6-hydroxychlorzoxazone.
2. To assess the potential for chlorzoxazone to alter the metabolic formation of SC-71597.

METHODS:



SC-66110



SC-71597

The ability of the eplerenone to inhibit the activity of CYP2E1 was evaluated in vitro in pooled human liver microsomes with final protein concentration of 0.100 mg/mL. Human liver microsomes diluted in 100 mM potassium phosphate buffer at pH 7.4 with chlorzoxazone concentrations, 0, 10.0, 25.0, 50.0, 100 and 250 μM) and eplerenone concentrations of 0.00, 0.10, 1.00, 50.0, 100, 200, and 300 μM. The samples were injected onto the ——— equipped with an appropriate ——— column. The *m/z* 431→211 product ions of SC-71597 were monitored.

Formation velocities of SC-71597 were calculated using the following formula:
V(nmoL/min/mg) = calculated ng/mL × 0.5mL / (431 ng/nmol)/20 min/0.05 mg protein

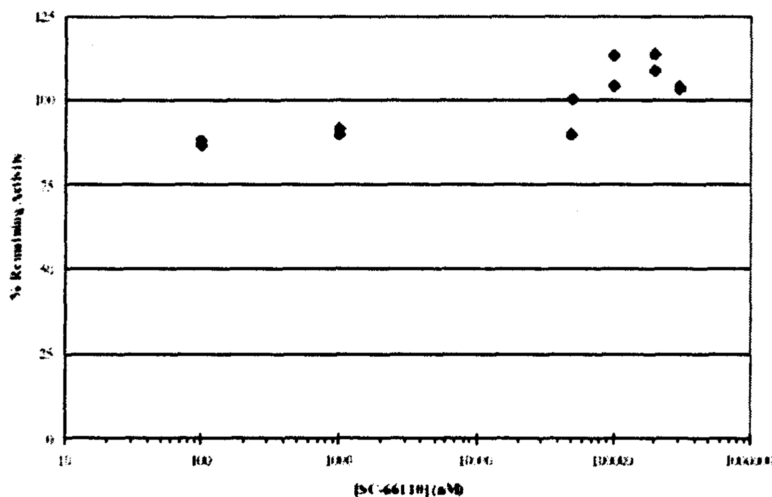
RESULTS:

Effect of Eplerenone on CYP2E1-Mediated Formation of 6-Hydroxychlorzoxazone
Incubation of eplerenone, at concentrations up to 300 μM, did not result in inhibition of CYP2E1-mediated formation of 6-hydroxychlorzoxazone when chlorzoxazone was incubated at either concentration (50.0 μM or 100 μM). The rate of 6-hydroxychlorzoxazone formation was slightly increased at the highest concentration of eplerenone (100, 200, and 300 mM).

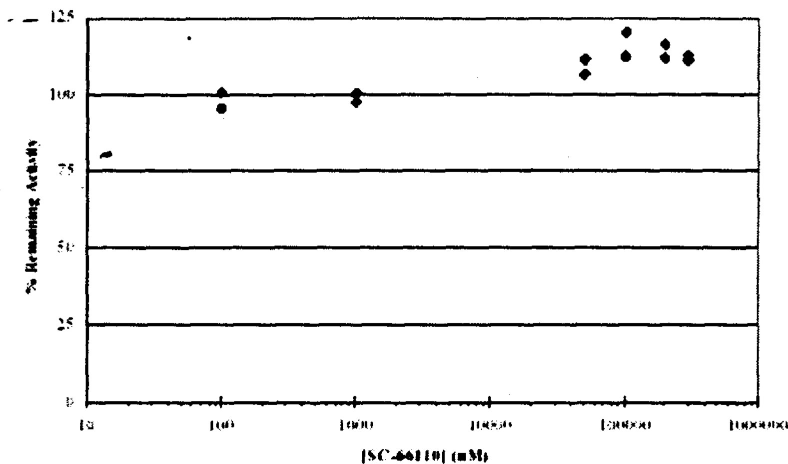
Table 1. Percent Remaining Activity Following Addition of Eplerenone To Incubations of Chlorzoxazone (50.0 µM and 100 mM)

Eplerenone Concentration (nM)	%Remaining Activity	
	50.0 µM Chlorzoxazone	100.0 µM Chlorzoxazone
100	88.1	101
	86.6	95.3
1000	89.7	97.2
	91.5	100
50000	100	112
	89.8	106
100000	113	120
	104	112
200000	114	112
	109	116
300000	103	112
	104	113
Positive Control	18.1	N/A
	17.0	N/A
	19.1	N/A

Effect of Eplerenone on the Formation of 6-Hydroxychlorzoxazone When Incubated with Chlorzoxazone (50.0 µM) In Vitro



Effect of Eplerenone on the Formation of 6-Hydroxychlorzoxazone When Incubated with Chlorzoxazone (100 µM) In Vitro



Effect of Chlorzoxazone on the Formation of SC-71597

The Ki estimated for chlorzoxazone inhibition of SC-71597 formation was 110 μM using a linear-mixed model of inhibition. The Ki of 110 μM is within the limit of chlorzoxazone plasma concentrations (~ 32-215 μM) obtained with normal chlorzoxazone doses of 250-750 mg, indicating potential interaction at therapeutic doses of chlorzoxazone when administered concomitantly with eplerenone.

CONCLUSIONS

Eplerenone did not inhibit CYP2E1-mediated formation of 6-hydroxychlorzoxazone for chlorzoxazone at concentrations up to 300 μM. Based on the results of the present study, eplerenone is not expected to interact with drugs such as enflurane, dichloromethane, halothane, and ethanol which are primarily cleared by CYP2E1.

Chlorzoxazone inhibited the formation of SC-71597 in a concentration dependent manner. The Ki estimated for chlorzoxazone inhibition was 110 μM, indicating possible reduction in eplerenone metabolism when administered concomitantly with chlorzoxazone.

APPEARS THIS WAY
ON ORIGINAL

IN VITRO DRUG-DRUG INTERACTION STUDIES WITH SC-66110 (EPLERENONE) AND DICLOFENAC

Document #: M2000394

OBJECTIVES:

To evaluate the potential for metabolic drug-drug interactions between eplerenone and CYP2C9 substrate diclofenac.

METHODS:

Evaluation of Diclofenac Disappearance:
The disappearance of diclofenac in the presence of eplerenone was investigated in vitro in human
liver microsomes. The concentrations for diclofenac were 5.00 and 25.0 μM concentrations. A volume of 25.0 μL microsomes was added to 425 mL 100 mM potassium phosphate buffer, pH 7.4. Eplerenone was added in a volume of 2 μL to final concentrations of 0.00, 1.00, 5.00, 25.0, 50.0, and 100 μM as appropriate, and diclofenac was added in a volume of 25.0 μL to reach final concentrations of 5.00 or 25.0 μM. The enzymatic reactions were initiated by addition of 25.0 μL of an NADPH (1.00 mM final concentration), and the samples were allowed to incubate at 37 °C for 15 minutes.

APPEARS THIS WAY ON ORIGINAL

Effect of Diclofenac on the Formation of SC-71597
A Ki quantifying inhibition of SC-71597 formation was estimated by incubating 5 eplerenone (substrate) concentrations with 6 concentrations of diclofenac (including zero). Briefly, human liver microsomes (25.0 μL) were added to 425 μL of 100 mM potassium phosphate buffer, pH 7.4 to achieve a final protein concentration of 0.100 mg/mL. A volume of 25 μL diclofenac was added to appropriate tubes. Diclofenac concentrations were 0.00, 20.0, 40.0, 100, 200, and 400 μM. Eplerenone was added to the appropriate suspensions to achieve the target concentrations of 25.0, 50.0, 100, 200, and 400 μM and the suspensions were allowed to equilibrate for approximately 3 minutes. The enzymatic reactions were initiated by the addition of NADPH (25.0 μL) so that the final concentration was 1.00 mM. Incubations were quenched after 15 minutes by the addition of the extraction solvent ethyl acetate.

Formation velocities of SC-71597 were calculated using the following formula:
V(nmol/min/mg) = calculated ng/mL x 0.5 mL /(431 ng/nmol)/15 min/0.05 mg protein

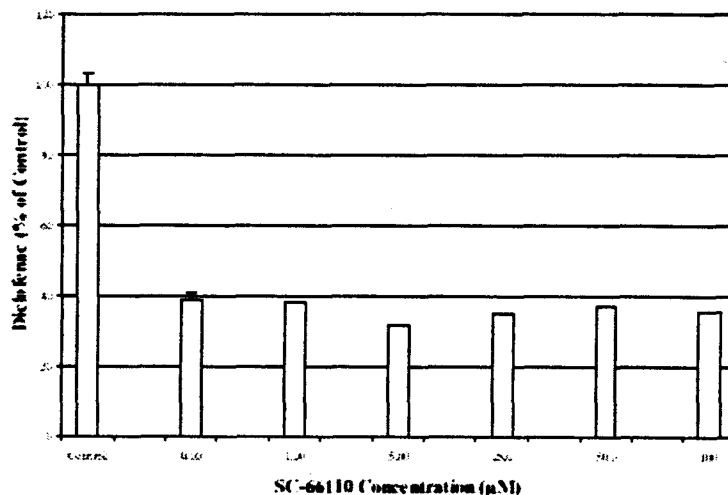
RESULTS:

Effect of Eplerenone on the Disappearance of Diclofenac:

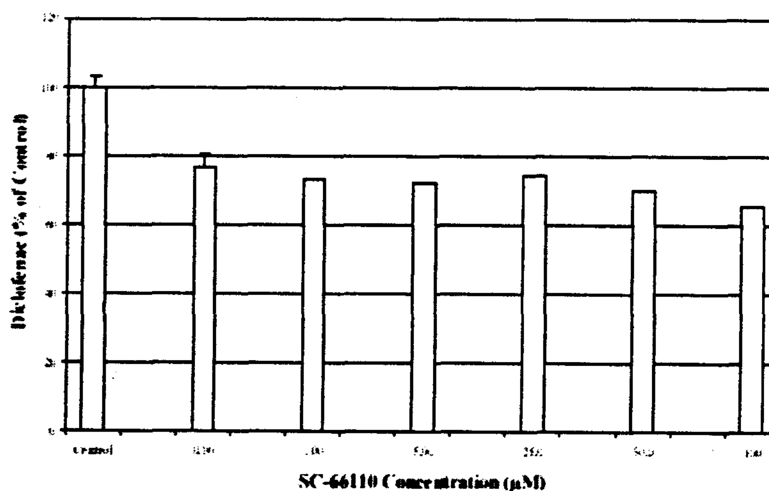
The addition of eplerenone at concentrations up to 100 μM did not affect the disappearance of diclofenac. In the absence of any eplerenone, mean diclofenac concentrations decreased by 38.8% and 76.6% after 15 minutes incubation with

diclofenac concentrations of 5.00 µM or 25.0 µM, respectively. This disappearance was dependent on the presence of the NADPH regenerating system indicating substantial P450 metabolism.

Effect of Eplerenone on the Depletion of Diclofenac (5.00 mM) In Vitro



Effect of Eplerenone on the Depletion of Diclofenac (25.0 mM) In Vitro



Effect of Diclofenac on the Formation of SC-71597

No effect was observed on the velocities of SC-71597 formation when diclofenac was added to the incubation mixture up to 400 µM. The Ki estimated for diclofenac inhibition of SC-71597 formation was 910 µM using a non-competitive model of inhibition. The

value for Ki estimated here (910 μM) significantly exceeds the plasma concentrations of diclofenac (~1.4-17 μM) obtained at therapeutically effective doses. The results of the present study suggest that a metabolic interaction between eplerenone and diclofenac is unlikely.

CONCLUSIONS

The addition of eplerenone at concentrations up to 100 μM did not affect the disappearance of diclofenac. Similarly, diclofenac concentration up to 400 μM did not have an effect on the formation of eplerenone metabolite SC-71597. A Ki of 910 μM was estimated using a non-competitive model of inhibition. The Ki value of 910 μM is significantly higher than the expected plasma concentrations of diclofenac obtained at therapeutic doses. In light of anticipated plasma concentrations of these drugs, the data suggest that *in vivo* drug-drug interactions between eplerenone and diclofenac are unlikely.

APPEARS THIS WAY
ON ORIGINAL

IN VITRO DRUG-DRUG INTERACTION STUDIES WITH SC-66110 (EPLERENONE) AND METHYLPHENIDATE

Document #: M2000333

OBJECTIVES:

1. To assess the potential for eplerenone to affect the in vitro clearance of methylphenidate.
2. To assess the potential for methylphenidate to alter the metabolic formation of SC-71597.

METHODS:

Evaluation of Methylphenidate Disappearance:
The metabolism of methylphenidate was initially investigated in pooled human liver microsomes, 2 mg/mL final concentration. A volume of 50 µL microsomes was added to 400 µL 100 mM potassium phosphate buffer pH 7.4. Eplerenone was added in a volume of 2.00 µL to final concentrations of 0, 1.00, 5.00, 25.0, 50 and 100 µM as appropriate. Methylphenidate was added to appropriate tubes in a volume of 25.0 µL to reach final concentrations of 750 or 1500 ng/mL. The enzymatic reactions were initiated after equilibration at 37 °C by adding 25.0 µL of NADPH regenerating system. The reactions were quenched after 3 hours incubation by addition of 0.300 mL of acetone and the samples were extracted and analyzed as described above. Since enzyme dependent disappearance of methylphenidate was not demonstrated in the initial experiments, the metabolism of methylphenidate was investigated by monitoring the formation of the acid metabolite ritalinic acid. Methylphenidate was added (2.00 µL) to 450 µL of chilled potassium phosphate buffer containing human liver microsomes (0.2 mg/mL). Final concentrations of methylphenidate were 100 and 500 ng/mL. The samples were incubated at 37 °C for 30 minutes. P450 dependent reactions were initiated by the addition of NADPH to a final concentration of 1.00 mM in a similar group of samples. Control incubations (n = 6) included samples incubated with heat inactivated microsomes and samples including the esterase inhibitor bis-nitrophenyl phosphate (BNPP, 20 µM). To investigate the potential for drug-drug interactions, eplerenone was added as appropriate to duplicate samples to final concentrations of 0, 1.00, 5.00, 25.0, 50.0, and 100 µM. Reactions were quenched after 30 minutes by the addition of 3.00 mL of ethyl acetate and the internal standard and the samples were extracted as described above.

Effect of Methylphenidate on the Formation of SC-71597:
Human liver microsomes (25.0 mL) were added to 450 µL of 100 mM potassium phosphate buffer pH 7.4 to achieve a final protein concentration of 0.1 mg/mL. Methylphenidate was added to appropriate tubes to concentrations of 0, 50.0, 75.0, 100, 250, and 500 µM. The concentrations of methylphenidate included were based on its solubility limitations. Eplerenone was added to the appropriate suspensions to achieve the target concentrations of 25.0, 50.0, 100, 200, and 400 µM and the suspensions were

allowed to equilibrate for approximately 3 minutes. The enzymatic reactions were initiated by the addition of NADPH (25.0 µL) so that the final concentration was 1.00 µM. Incubations were quenched after 15 minutes by the addition of the extraction solvent ethyl acetate. The samples were injected onto the equipped with an appropriate column. The *m/z* 431→211 product ions of SC-71597 were monitored.

Formation velocities of SC-71597 were calculated using the following formula:
V(nmol/min/mg) = calculated ng/mL x 0.5mL / (431 ng/nmol)/15 min/0.05 mg protein

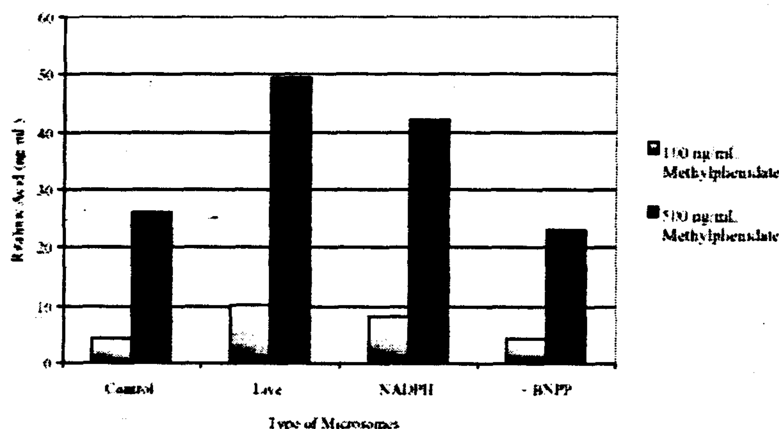
RESULTS:

Effect of Eplerenone on the Disappearance of Methylphenidate and Formation of Ritalinic Acid

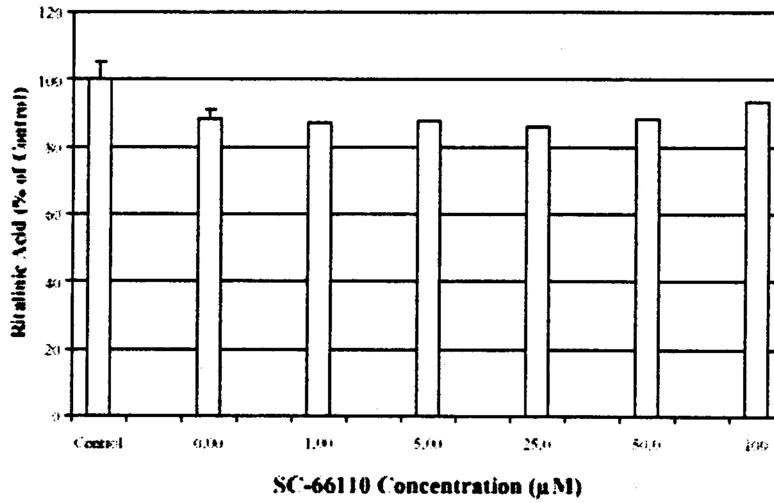
Methylphenidate disappearance could not be related to enzymatic activity. Consequently, potential interactions with eplerenone were investigated by monitoring the metabolic formation of ritalinic acid. When methylphenidate was incubated 30 minutes at either 100 or 500 ng/mL, formation of ritalinic acid was observed. The formation was not totally dependent on enzyme activity since 4.26 ± 0.500 and 26.0 ± 2.47 ng/mL ritalinic acid were found in respective suspensions incubated with heat-inactivated microsomes. However, the amount of ritalinic acid formed was approximately two fold higher in suspensions incubated with enzymatically active "live" microsomes (10.1±0.970 and 49.4±2.33 ng/mL, respectively). Inclusion of BNPP in incubation suspensions completely inhibited the enzymatic formation of ritalinic acid. No additional activity was observed when NADPH was included in the incubation mixtures. These observations suggest that the formation of ritalinic acid is not dependent on the activity of P450 enzymes but is more likely related to esterase activity which is inhibited by BNPP.

Addition of eplerenone to incubation suspensions at concentrations up to 100 µM had no effect on the formation of ritalinic acid.

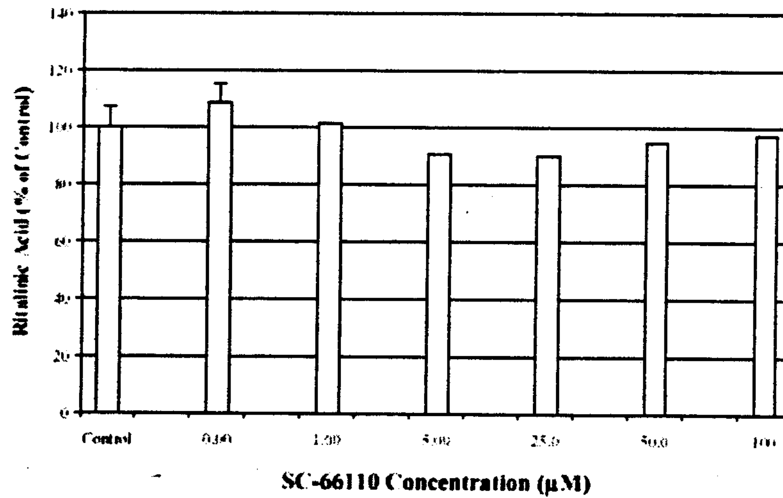
Formation* of Ritalinic Acid (30 Minute Incubation)



Effect of Eplerenone on the Formation of Ritalinic Acid In Vitro (Methylphenidate 100 ng/mL)



Effect of Eplerenone on the Formation of Ritalinic Acid In Vitro (Methylphenidate 500 ng/mL)



Effect of Methylphenidate on the Formation of SC-71597

The Ki estimated for methylphenidate inhibition of SC-71597 formation was 930 µM using a noncompetitive model of inhibition. The Ki of 930 µM substantially exceeds the anticipated plasma concentrations of methylphenidate which are typically 70-100 nM. However, concentrations of ritalinic acid have been reported to exceed methylphenidate concentrations by 50-100 fold in normal adults. The effect of ritalinic acid on formation of SC-71597 was not assessed in the present study. Treatment with methylphenidate has been noted to result in interactions with some anticoagulants and with phenytoin, both substrates of CYP2C9 and CYP2C19. The results of the present study indicate that eplerenone is not expected to interact with the formation of methylphenidate to ritalinic acid. Similarly, methylphenidate is unlikely to alter clearance of eplerenone through pathways involving SC-71597.

CONCLUSIONS

Eplerenone concentrations up to 100 µM had no effect on the formation of ritalinic acid. The rate of SC-71597 formation was decreased in a concentration dependent manner by methylphenidate. The Ki estimated using a noncompetitive model of inhibition was 930 µM, however this concentration far exceeds methylphenidate plasma concentrations obtained at therapeutic doses.

APPEARS THIS WAY
ON ORIGINAL

DETERMINATION OF POTENTIAL FOR PHARMACOKINETIC INTERACTIONS BETWEEN SC-66110 (EPLERENONE) AND LOSARTAN

Document #: M3198199

Losartan is converted, in part, to an active carboxylic acid metabolite, losartan carboxylic acid, which is responsible for most of the angiotensin II receptor antagonism following losartan treatment. The carboxylic acid metabolite is formed by both CYP2C9 and by CYP3A4.

OBJECTIVES:

To determine the effect of eplerenone and losartan on rate of metabolism of either drug in vitro by human liver microsomes.

METHODS:

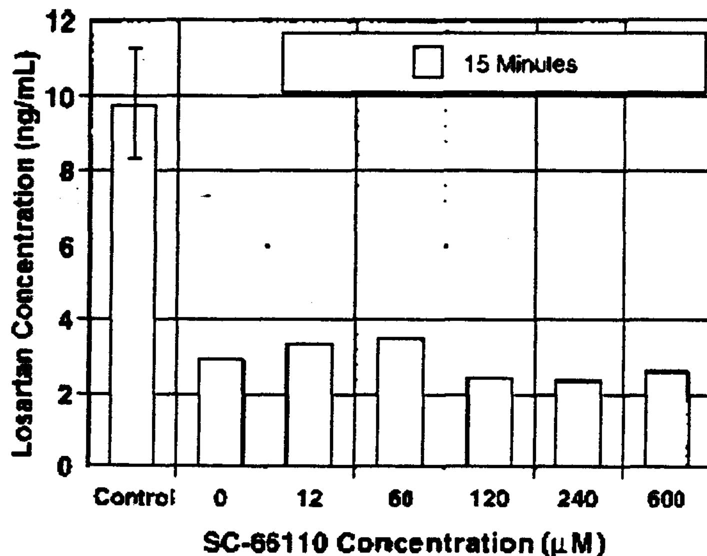
Six concentrations of eplerenone and 8 concentrations of losartan were incubated with human liver microsomes (HLM) at 37°C under air in reaction mixtures fortified with an NADPH-generating system. The concentrations of eplerenone ranged from 12 to 600 μM and the concentrations of losartan ranged from 0.0109 to 10.9 μM.

RESULTS:

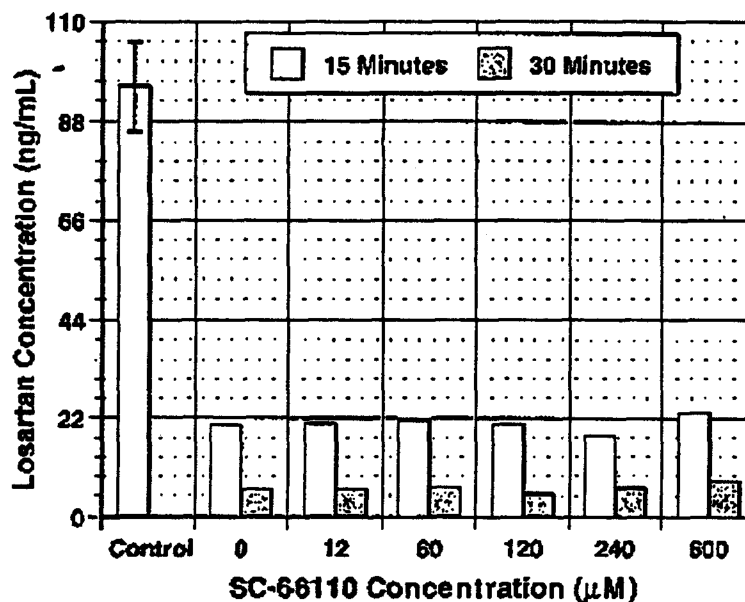
Effect of Eplerenone on Losartan Metabolism in Human Liver Microsomes

The results for both the disappearance of losartan and for the formation of losartan carboxylic acid indicate little if any effect of eplerenone on losartan metabolism by human liver microsomes. Differences between disappearance of losartan in the absence and presence of eplerenone were generally less than 10% and showed no clear dose-response. Likewise, formation of losartan carboxylic acid was not appreciably decreased, at either 15 minutes or 30 minute of incubation.

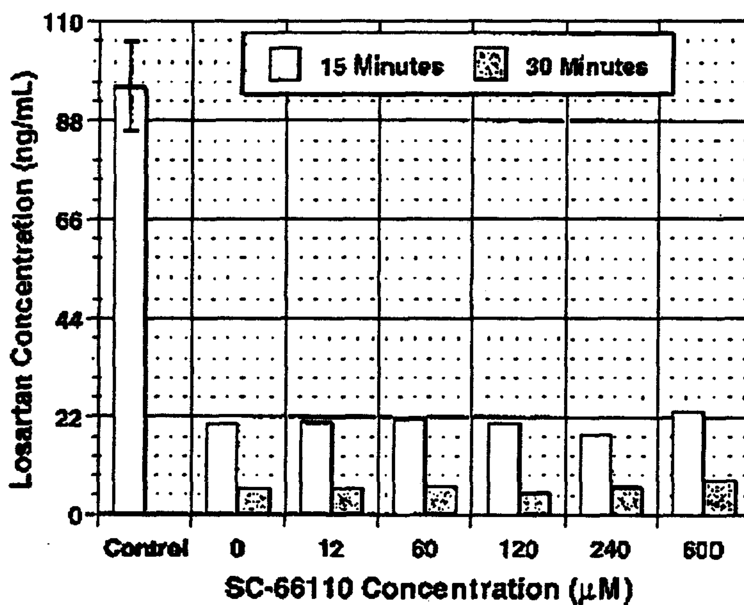
Figure 1. Effect of Eplerenone on the Depletion of Losartan (0.0327 mM) During Incubation with Human Liver Microsomes



Effect of Eplerenone on the Depletion of Losartan (0.109 mM) During Incubation with Human Liver Microsomes



Effect of Eplerenone on the Depletion of Losartan (0.327 mM) During Incubation with Human Liver Microsomes



Effect of Eplerenone on the Depletion of Losartan (1.09 mM) During Incubation with Human Liver Microsomes