

Eplerenone: A Selective Aldosterone Receptor Antagonist (SARA)

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ABSTRACT

Aldosterone, the final product of the renin-angiotensin-aldosterone system (RAAS), is a mineralocorticoid hormone that classically acts, via the mineralocorticoid (aldosterone) receptor, on epithelia of the kidneys, colon, and sweat glands to maintain electrolyte homeostasis. Aldosterone has also been shown to act at nonepithelial sites where it can contribute to cardiovascular disease such as hypertension, stroke, malignant nephrosclerosis, cardiac fibrosis, ventricular hypertrophy, and myocardial necrosis. Although angiotensin-converting enzyme (ACE) inhibitors and angiotensin type 1 (AT₁) receptor antagonists act to suppress the RAAS, these agents do not adequately control plasma aldosterone levels — a phenomenon termed “aldosterone synthesis escape.” Spironolactone, a nonselective aldosterone receptor antagonist, is an effective agent to suppress the actions of aldosterone; its use is, however, associated with progestational and antiandrogenic side effects due to its promiscuous binding to other steroid receptors. For these reasons, eplerenone — the first agent of a new class of drugs known as the selective aldosterone receptor antagonists (SARAs) — is under development. In rodent models, eplerenone provides marked protection against vascular injury in the kidney and heart. In phase II clinical trials, eplerenone demonstrates 24-h control of blood pressure with once or twice daily dosing, and is safe and well tolerated in patients with heart failure when given with standard of care agents. Pharmacokinetic studies reveal that eplerenone has good bioavailability with low protein binding, good plasma exposure, and is highly metabolized to inactive metabolites and excreted principally in the bile. Eplerenone is well tolerated in acute and chronic safety pharmacology studies. Ongoing phase III trials of eplerenone in the treatment of hypertension and heart failure are underway. These studies will extend our understanding

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of selective aldosterone receptor antagonism in the treatment of chronic cardiovascular disease.

INTRODUCTION

Numerous studies have demonstrated an important role of the renin-angiotensin-aldosterone system (RAAS) in cardiovascular disease (8,24,30,31,36). In an attempt to abrogate the effects of the RAAS, angiotensin-converting enzyme (ACE) inhibitors have been used in the treatment of cardiovascular diseases such as hypertension, (56) diabetic nephropathy (27) and congestive heart failure (4,5,20,33). More recently, the angiotensin type I (AT₁) receptor antagonists have been shown to have utility in some of these same disease states (1,32,34) confirming the utility of pharmacologic intervention in this system. However, neither ACE inhibitors nor AT₁ receptor antagonists chronically reduce plasma aldosterone levels, a phenomenon termed "aldosterone synthesis escape," as demonstrated by the Randomized Evaluation of Strategies for Left Ventricular Dysfunction (RESOLVD) trial (30) thus leaving potential detrimental effects of the final hormone in this system unabated. The most compelling evidence supporting this hypothesis is from the recent publication of the Randomized Aldactone Evaluation Study (RALES) (36). In this study, patients with New York Heart Association (NYHA) class III and IV heart failure were treated with the nonselective aldosterone receptor antagonist, spironolactone, or placebo in addition to standard of care, which included ACE inhibitors, digitalis, and diuretics. The study was concluded prematurely due to a highly significant benefit of aldosterone receptor antagonism on mortality (30% reduction vs. placebo), demonstrating that blocking the actions of aldosterone has clinical benefit even when other RAAS blocking agents are employed.

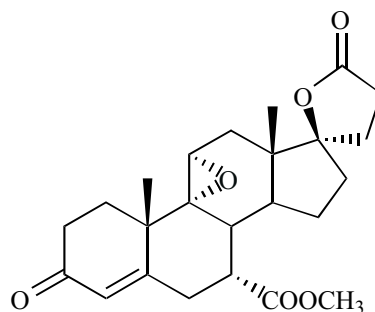
Aldosterone is a mineralocorticoid hormone synthesized in the adrenal glomerulosa. Classically, aldosterone acts on epithelia of the kidney, colon, and sweat glands via the mineralocorticoid receptor (MR) to promote the retention of sodium and the excretion of potassium, thus playing a critical role in electrolyte homeostasis. Abnormal activity of this hormone can lead to the development of edematous states. In addition to the classical epithelial actions, aldosterone binds to MR in nonepithelial tissues, such as the heart, brain, and blood vessels, where it is capable of mediating several pathophysiological actions (2, 15,26,28,46–48,50,51). For example, aldosterone has been reported to mediate hypertension in rats via central nervous system actions (21,22,57), and lead to stroke (42), malignant nephrosclerosis (42,43), cardiac fibrosis (49,55), ventricular hypertrophy (14,25, 54), and myocardial necrosis (45).

Spironolactone (Aldactone®) is the only clinical agent that directly blocks the actions of aldosterone. Although spironolactone is an effective aldosterone receptor antagonist, it is not without limitations due to its promiscuous steroid receptor binding, which result in antiprogesterational and antiandrogenic side effects. These are most commonly manifested as gynecomastia, abnormal menstrual cycles, and impotence, limiting its use by physicians in the chronic treatment of disease. In light of the important role of aldosterone in cardiovascular disease and the side effects that accompany the use of spironolactone, eplerenone, a selective aldosterone receptor antagonist (SARA), is under development (10).

CHEMISTRY

The chemical structure of eplerenone, pregn-4-ene-7,21-dicarboxylic acid, 9,11-epoxy-17-hydroxy-3-oxo, γ -lactone, methyl ester (7 α , 11 α , 17 α) (SC-66110), is shown in Fig. 1. It is an odorless, white to off-white solid, with a molecular weight of 414.50 and empirical formula of C₂₄H₃₀O₆.

Eplerenone is soluble in dichloromethane and acetonitrile, sparingly soluble in methyl ethyl ketone and methanol, and slightly soluble in ethanol. Eplerenone has no ionizable groups. The aqueous solubility of eplerenone is low and is independent of pH. The stability of eplerenone aqueous solution was examined at pH values of 2, 5, 7, and 9, and was found to be pH-dependent, with maximum stability at low pH values. The solution stability at a pH of 9 was poor. The major degradation pathways *in vitro* at low and high pH values were hydrolysis of the acetate ester and opening of the lactone ring, respectively. There appeared to be an equilibrium between the lactone and the open lactone ring product (SC-70303). At pH values of 7 and 9, formation of the open lactone ring dominated the degradation rate. At all pH values examined, fluorescent light caused additional degradation.



SC-66110
(Eplerenone)
C₂₄H₃₀O₆
(414.50)

FIG. 1. Chemical structure of eplerenone (SC-66110).

PHARMACOLOGY

Receptor Binding Selectivity *in Vitro*

The purpose of these experiments was to compare the binding characteristics of eplerenone and spironolactone to the mineralocorticoid and other steroid receptors. *In vitro* binding studies were performed using standard cytosolic preparations of appropriate tissues from rats or rabbits. The ability of eplerenone and spironolactone to antagonize steroid receptors was studied by comparing the binding affinity of the test compounds (i.e., eplerenone and spironolactone) to a standard agonist of the receptor. Thus, the greater the ratio, the more avid the binding of the test compound. The data in Table 1 indicate that, *in vitro*, spironolactone is a more potent antagonist of the MR than eplerenone; however, unlike spironolactone, eplerenone binds poorly to other steroid receptors, indicating that eplerenone is a SARA (9,37).

Mineralocorticoid Receptor Binding *in Vivo*

The inhibition of aldosterone binding to the MR by eplerenone was examined *in vivo* by measuring radiolabeled aldosterone bound to kidney receptors in adrenalectomized rats pretreated with eplerenone. Spironolactone was also studied for comparison. The results

indicated that, in this species, oral potency of eplerenone, as an inhibitor of aldosterone binding renal MR, was similar or slightly higher than that of spironolactone ($ED_{50} = 0.8$ vs. 1.7 mg/kg, respectively, $p > 0.05$). Thus, despite the lower *in vitro* binding affinity (Table 1), *in vivo*, as an aldosterone antagonist, eplerenone is as potent or slightly more potent than spironolactone (Table 2) (9).

Effect of Plasma Proteins on Receptor Binding Affinity *in Vitro*

In vitro studies indicated that spironolactone has approximately a 20-fold greater affinity for the MR than eplerenone, yet *in vivo* studies indicated similar MR binding (9). In order to reconcile these findings, the *in vitro* binding of eplerenone and spironolactone was determined in the presence of increasing concentrations of plasma (as a percent of the total incubation medium) using identical methods as discussed above. The apparent IC_{50} of spironolactone increased nearly three-fold in the presence of 20% plasma. In contrast, increasing plasma concentrations had no such effect on the binding affinity of eplerenone (Table 3). Therefore, the observed eplerenone and spironolactone *in vivo* binding, despite

TABLE 1. Relative binding affinities of eplerenone and spironolactone for steroid receptors *in vitro**

Receptor (standard)	Spironolactone	Eplerenone
Mineralocorticoid (aldosterone)	$1.1 \times 10^{-1} \pm 36.4\%$ (7)	$5.1 \times 10^{-3} \pm 6.5\%$ (7)
Glucocorticoid (dexamethasone)	$1.8 \times 10^{-3} \pm 16.1\%$ (4)	$1.8 \times 10^{-4} \pm 22.2\%$ (4)
Androgen (methylthienolone-1)	$9.1 \times 10^{-3} \pm 16.5\%$ (7)	$7.6 \times 10^{-5} \pm 26.3\%$ (6)
Progesterone (progesterone-1)	$7.0 \times 10^{-3} \pm 11.0\%$ (5)	$<5 \times 10^{-5}$ (5)

Affinities are expressed as the ratio of $IC_{50s}/IC_{50t} \times 100$, where s is the standard used and t is the test compound, i.e., the higher the number, the greater affinity for the receptor. The S.E.M.s are expressed in percentage of the mean for easier comparison. Numbers in parenthesis, number of determinations.

* Due to poor solubility in the assay conditions, this value is difficult to estimate.

Adapted from refs. 9,37.

TABLE 2. Inhibition of $[H^3]$ aldosterone binding by eplerenone and spironolactone: *in vivo* tests

Dose (mg/kg)	Eplerenone	Spironolactone
0.3	73.5 ± 4.7 (6)	—
1.0	$47.5 \pm 4.0^*$ (8)	64.5 ± 6.5 (8)
3.0	$14.0 \pm 3.1^*$ (6)	39.3 ± 9.5 (6)
10.0	8.2 ± 0.5 (6)	5.2 ± 1.7 (6)

The results are expressed in percentage of the control value. Number in parenthesis represent number of determinations. * $P < 0.05$ vs. spironolactone. Adapted from ref. 9.

TABLE 3. Effect of rat plasma on apparent IC_{50} values (nM) for inhibition of binding of $[H^3]$ aldosterone by eplerenone or spironolactone to mineralocorticoid receptors *in vitro*

Rat Plasma	Spironolactone	Eplerenone
0 %	2.2	128.4
5 %	3.5	112.7
20 %	5.2	105.7

Binding affinity of spironolactone and eplerenone (SC-66110) for the rat colon mineralocorticoid receptor was examined in the presence of increasing concentrations of rat plasma. Values represent IC_{50} calculated from the binding curves obtained from each experiment.

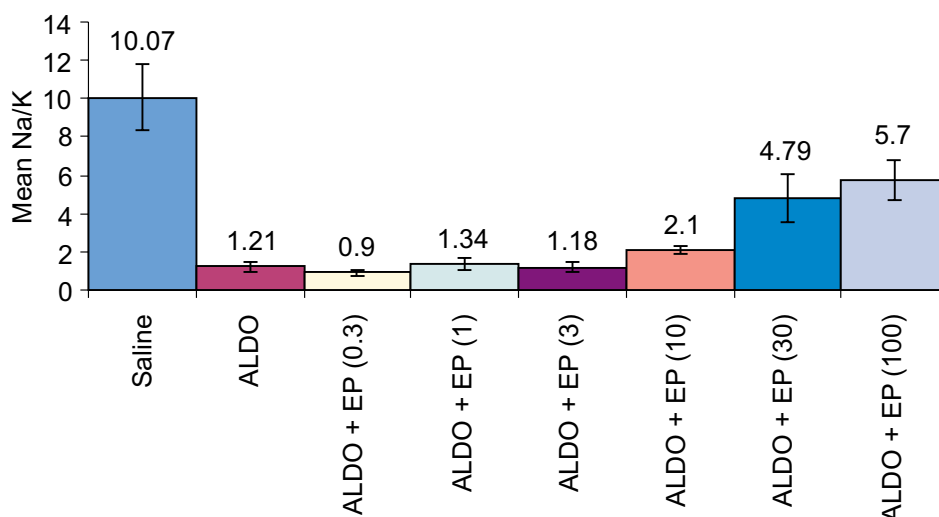


FIG. 2. Effect of aldosterone antagonists administered by oral gavage in the rat Kagawa assay. ALDO, aldosterone; EP, eplerenone; numbers in parentheses represent the dose of antagonist administered (in mg/kg); number of animals for each group was between 8 and 11 (11).

superior *in vitro* MR affinity of spironolactone, may be partly explained by more avid plasma protein binding of spironolactone as compared to eplerenone.

Effect of Eplerenone on *in Vivo* Kidney Responsiveness to Aldosterone

The antimineralocorticoid pharmacologic activity of eplerenone was determined in saline-loaded adrenalectomized rats treated with aldosterone. Eplerenone was given by oral gavage 30 minutes before the aldosterone injection. Urinary Na^+ , K^+ , and water excretion were measured. The results are expressed as the ratio of excreted urinary Na^+ to K^+ , (Na^+/K^+) \pm S.E.M. (Fig. 2). Aldosterone administration resulted in a predicted increase in the urinary excretion of K^+ and retention of Na^+ , indicated by a marked decrease in the Na^+/K^+ ratio (10.07 vs. 1.21). Eplerenone dose-dependently reversed the effect of aldosterone, such that the maximum dose studied (100 mg/kg) reversed the effect by approximately 57%. These data indicate that eplerenone is an orally active MR antagonist, which can reverse the renal actions of aldosterone (Fig. 2) (11).

Effect of Eplerenone on Renal Injury in the Stroke-Prone Spontaneously Hypertensive Rats (SHRSP)

Previous studies have demonstrated that the nonselective aldosterone receptor antagonist, spironolactone, reduced proteinuria and vascular injury in SHRSP (42). More recently, the ability of eplerenone to mimic the protective effect of spironolactone in rats was established (44). Saline-drinking SHRSP ($n = 8$) were treated for 5 to 6 weeks with either eplerenone (100 mg/kg/d, p.o. b.i.d.) or vehicle, starting at 8 weeks of age.

Treatment with eplerenone prevented the development of proteinuria and renal lesions. These effects occurred despite the absence of major reductions in blood pressure and were similar to the previous results reported with spironolactone in SHRSP (42). In additional experiments, captopril-treated (50 mg/kg/d, p.o.), saline-drinking SHRSP were infused with either vehicle or angiotensin II (25 ng/min, s.c.) for 2 weeks, starting at 9.3 weeks of age (44). To evaluate the role of aldosterone as part of the RAAS in the development of renal injury, angiotensin II-infused rats were treated with eplerenone (100 mg/kg/d, p.o. b.i.d.) or placebo. All experimental groups had similar systolic blood pressure. Captopril provided marked protection against glomerular and vascular damage and proteinuria. However, if rats treated with captopril also received angiotensin II, plasma aldosterone became elevated and the protective effects of captopril were eliminated, suggesting that the protective effects of captopril are a result, at least in part, of inhibition of angiotensin II production. Interestingly, the reestablished pathology induced by angiotensin II infusion in the captopril-treated animals was substantially attenuated by treatment with the SARA eplerenone. These data demonstrate that the renal pathology evident in this model is mediated through the RAAS, and that aldosterone is the primary mediator of the injury.

Effect of Eplerenone on Cardiovascular Injury in L-NAME/Ang II/NaCl Hypertensive Rats

To explore the potential beneficial role of eplerenone in end-organ damage, an experimental rat model was studied that combines elevated blood pressure, moderately high salt intake, activation of the RAAS with angiotensin II (Ang II), and blunted nitric oxide production using N^o-nitro-L-arginine methyl ester (L-NAME). The specific purpose of this study was to test whether reduction of mineralocorticoids by either adrenalectomy or pharmacologic antagonism with eplerenone could prevent cardiovascular injury and whether aldosterone replacement in adrenalectomized rats would restore damage. Urinary protein excretion (24 h) measured at the end of the 2-week treatment period was normal in the NaCl group. Treatment with L-NAME/Ang II/NaCl markedly increased blood pressure and urinary protein excretion. Eplerenone treatment and adrenalectomy prevented the development of proteinuria in animals receiving L-NAME/Ang II/NaCl treatment but did not reduce blood pressure (Fig. 3). Administration of aldosterone to adrenalectomized rats completely restored the effects of L-NAME/Ang II/NaCl treatment on proteinuria. Histopathologic evaluation of the kidneys demonstrated that, whereas renal arteriopathy was not found in kidneys from NaCl-drinking controls, animals receiving L-NAME/Ang II/NaCl treatment had severe renal vascular damage involving primarily arcuate and interlobular arteries and arterioles. These vessels demonstrated fibrinoid necrosis of the vascular wall with medial thickening and proliferation of the perivascular connective tissue. A few isolated glomeruli had areas of focal thrombosis. Renal arteriopathy tended to be reduced in animals receiving eplerenone treatment, although there was no statistically significant difference. Adrenalectomy significantly reduced the renal arteriopathy induced by L-NAME/Ang II/NaCl treatment to levels that were not significantly different from NaCl-drinking controls. When aldosterone was infused into adrenalectomized L-NAME/Ang II/NaCl-treated rats, damage was significantly in-

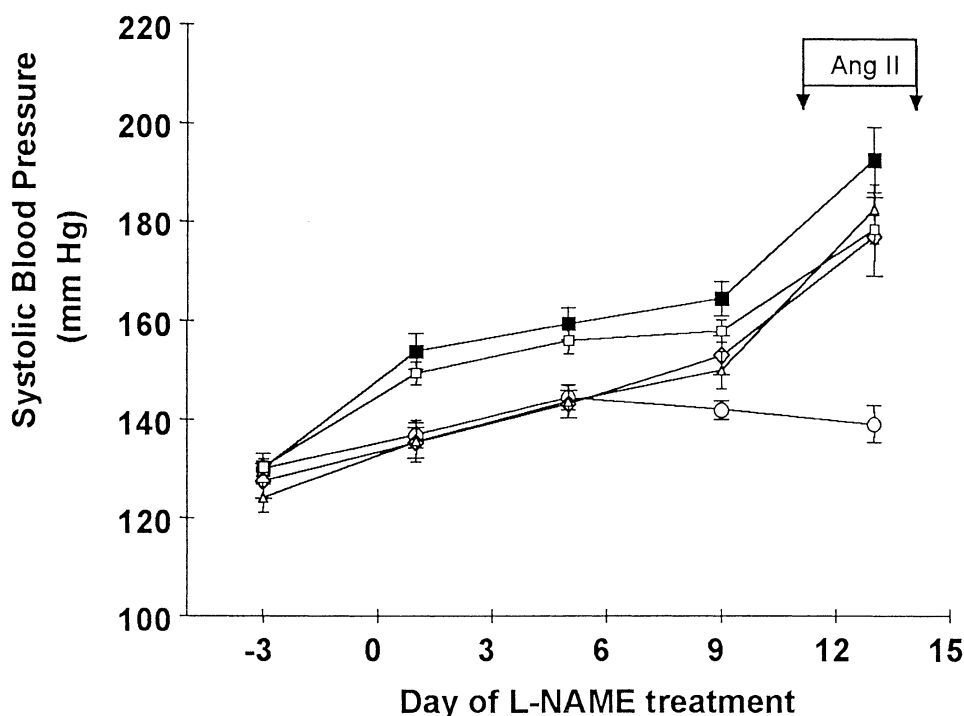


FIG. 3. Systolic blood pressure. Tail-cuff measurements of systolic blood pressure obtained before and after initiation of L-NAME treatment on days 1, 5, 9, and 13. Ang II was infused s.c. starting on day 11. Rats were killed on day 14. Groups: NaCl (Δ); L-NAME/Ang II/NaCl (\circ); L-NAME/Ang II/NaCl plus eplerenone (\diamond); L-NAME/Ang II/NaCl plus adrenalectomy (\square); L-NAME/Ang II/NaCl plus ADX/aldosterone (\blacksquare). $P < 0.01$ in all groups vs. NaCl. Values are means \pm S.E.M. Adapted with permission from ref. 44.

creased. Thus, eplerenone reduced proteinuria and produced a trend toward reduction of renal vascular injury measured histopathologically in this model of severe cardiovascular injury. These protective mechanisms are independent of blood pressure reduction (Fig. 3). At the level of the heart, L-NAME/Ang II/NaCl-treated rats developed severe coronary vascular injury and myocardial necrosis. Histopathological scoring of cardiac injury in response to treatment with L-NAME/Ang II/NaCl was markedly reduced in those animals in which eplerenone was chronically administered or adrenalectomy was performed (Fig. 4). These two groups demonstrated levels of myocardial necrosis that were similar to those observed in the NaCl-drinking controls. The protective effect of adrenalectomy was completely reversed by infusion of aldosterone. Thus, both eplerenone and adrenalectomy are effective in reducing myocardial injury in this model, without significant reduction in blood pressure, suggesting that in this model of hypertension, the damaging cardiovascular effects of L-NAME/Ang II/NaCl treatment are mediated, at least in part, by aldosterone. The primary protective effect of eplerenone in this model was a reduction in medial fibrinoid necrosis in small coronary arteries and arterioles and a reduction in subsequent tissue necrosis (38–41, 45).

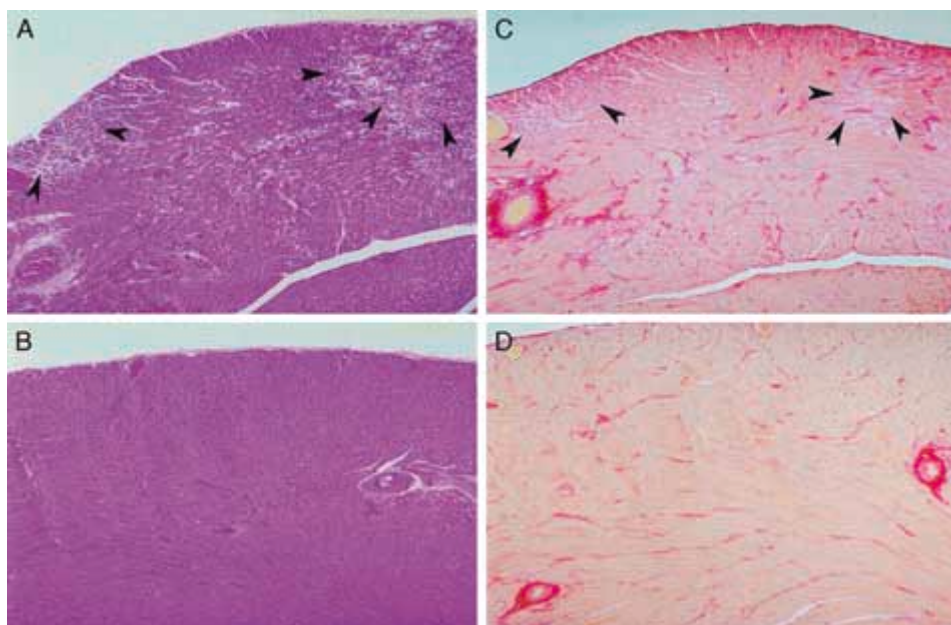


FIG. 4. Cardiac histopathology. **A**, Representative myocardial necrotic lesions (arrowheads) induced by L-NAME/Ang II/NaCl treatment (hematoxylin and eosin; magnification, $\times 40$). These lesions were observed in both the left and right ventricles. **B**, Myocardium of an animal receiving L-NAME/Ang II/NaCl treatment in the presence of the mineralocorticoid receptor antagonist eplerenone, showing no necrotic lesions. This figure is also representative of histologic sections from control, NaCl-drinking rats, and from adrenalectomized animals receiving L-NAME/Ang II/NaCl. **C** and **D**, Staining of the hearts from **A** and **B** with the collagen-specific dye Sirius red did not reveal increased interstitial or reparative collagen deposition, even in areas where myocardial necrosis had occurred (arrowheads in **C**). Adapted with permission from ref. 45.

Protective Effect of Eplerenone in Myocardial Tissue Healing

Myocardial infarction (MI) initiates tissue remodeling that includes infarct healing, maladaptive fibrosis, and left ventricular dilation, but is not characterized by systemic elevation of plasma aldosterone, unlike the models discussed previously. The role of aldosterone in post-MI tissue healing and remodeling was evaluated in Sprague–Dawley rats using eplerenone. Infarct healing and left ventricular remodeling were evaluated at 3, 7, and 28 days following MI by determining the diastolic pressure/volume relationship of the left ventricle, the infarct thinning ratio, and the collagen volume fraction. Eplerenone did not impact reparative collagen deposition as evidenced by a similar collagen volume fraction in the infarcted myocardium between eplerenone- and vehicle-treated groups at 7 and 28 days post-MI. In addition, the thinning ratio, an index of infarct expansion, was comparable between the eplerenone- and vehicle-treated animals at 7 and 28 days post-MI. In fact, eplerenone demonstrated a protective effect against maladaptive processes. At 28 days post-MI, the pressure/volume relationship of the left ventricle was shifted toward the noninfarcted control hearts, and the collagen volume fraction in the viable myocardium was reduced in animals that received eplerenone compared with ve-

hicle-treated animals, indicating that eplerenone may reduce left ventricular remodeling and reactive fibrosis, respectively. Thus, eplerenone does not retard infarct healing, but rather protects against maladaptive responses following MI in this model (12).

PHARMACOKINETICS

Preclinical

Pharmacokinetic studies of eplerenone were conducted in dogs following i.v., oral, and rectal dosing (15 mg/kg) and following intragastric, intraduodenal, intrajejunal, and intracolonic dosing (7.5 mg/kg). After oral administration, the systemic availability of eplerenone was 79.2%. Systemic availabilities following administration via other routes were similar to that following oral administration. The half-life and plasma clearance of eplerenone were 2.21 h and 0.329 L/kg/h, respectively. Plasma concentrations of SC-70303 were lower than eplerenone concentrations regardless of the route of administration. The [^{14}C]AUC in red blood cells was approximately 64 and 68% of the plasma AUC for i.v. and oral doses. Percentages of the dose excreted as total radioactivity in urine and feces were 54.2 and 40.6%, respectively, after i.v. administration, and 40.7 and 52.3%, respectively, after oral administration. The percentages of the dose excreted in urine and feces as eplerenone were 13.7 and 2.5%, respectively, after i.v. administration, and 2.1 and 4.6% after oral administration, respectively. Approximately 11 and 15% of the doses were excreted as the open form (SC-70303) following i.v. and oral doses. Eplerenone was rapidly and efficiently absorbed throughout the gastrointestinal tract, resulting in a good systemic availability. The drug did not preferentially accumulate in red blood cells. Eplerenone was extensively metabolized; however, first-pass metabolism after oral and rectal administration was minimal (6).

Clinical

Clinical pharmacokinetic studies assessed the disposition kinetics of eplerenone in man and the major CYP450 isozyme responsible for the biotransformation of eplerenone. Subjects received a single oral 100-mg dose of [^{14}C]eplerenone (7.5 μCi) as an oral solution. The *in vitro* metabolic pathway of eplerenone was evaluated utilizing human liver microsomes and heterologously expressed CYP450 proteins. Results showed that the primary drug-related material in the plasma was unchanged eplerenone. Protein binding of eplerenone was 49%. A total of $66.6 \pm 1.1\%$ (mean \pm S.D.) and $32.0 \pm 1.3\%$ of the administered [^{14}C] dose was recovered in the urine and feces, respectively. Eplerenone was extensively metabolized, with $<1.7\%$ of the dose excreted unchanged in urine and 0.8% in feces. All metabolites identified were inactive. The inactive open lactone ring form of eplerenone, SC-70303, constituted $5.0 \pm 0.5\%$ and $2.5 \pm 0.3\%$ of the dose in urine and feces, respectively. [^{14}C]Eplerenone was readily absorbed, with a maximum concentration of 1721 ± 290 ng/mL, a time to maximum concentration of 1.3 h, a half-life of 3.8 ± 1.1 h, and an $\text{AUC}_{(0-\infty)}$ value of 9537 ± 3201 ng/mL/h. *In vitro* studies indicated that the primary hydroxylated metabolite of eplerenone was formed predominantly via the CYP3A4 isozyme. Eplerenone appears to be rapidly absorbed and eliminated, with a half-life of about

4 h. Its primary route of elimination is via metabolism, with inactive metabolites formed predominantly via the CYP3A4 isozyme and eliminated in the urine (53).

TOXICOLOGY

Acute Toxicity

Single-dose toxicology studies of eplerenone in the mouse, rat, and dog indicate a low order of acute toxicity. There were no deaths at the highest dose evaluated in any species (up to 1000 mg/kg in mice and 2000 mg/kg in rats and dogs).

Repeated-Dose Toxicity

Eplerenone was well tolerated in all repeated-dose toxicity studies in rats (with durations from 8 days to 1 year and dosages as high as 1000 mg/kg/d). Findings of urine electrolyte changes, increased plasma aldosterone levels, and hypertrophy of the adrenal zona glomerulosa were associated with the predicted exaggerated pharmacology of aldosterone receptor antagonism. The only apparent toxic effect in rats was an increased incidence of chronic progressive nephropathy (CPN), a common spontaneous aging kidney disease of rats thought not to be predictive of clinical findings, which occurred after 13 weeks or more of dosing with systemic exposures seven-fold higher than the predicted human efficacious exposure. CPN is characterized by increased protein leakage through the glomerulus and by progressive kidney lesions comprising thickened glomerular and tubular basement membranes, dilated tubules with protein casts, regenerative tubular epithelium and, in advanced cases, tubular mineral deposits, interstitial inflammation and scarring. Other changes in the rat appear to be associated with the induction of hepatic CYP450-3A, the "steroid-inducible" form of CYP450, and uridine diphosphate glucuronosyl transferase (UDPGT), an enzyme that conjugates thyroxine for biliary excretion. This induction resulted in increased liver weight, elevation of thyroid stimulating hormone (TSH) with trophic effects on the thyroid gland, and increases in some serum constituents such as cholesterol, triglyceride, and total protein.

Studies of eplerenone in the beagle dog showed no overt toxicity at dosages up to 100 mg/kg/d for up to 1 year. At 300 mg/kg/d, a few dogs showed toxicity 2 to 4 weeks after the initiation of dosing; signs included electrolyte disturbances, dehydration, and weight loss. Exaggeration of the pharmacologic action of eplerenone in dogs was manifest as dosage-related increases of serum aldosterone and hypertrophy of the zona glomerulosa of the adrenal. In all dog studies of 13 weeks or more the principal adverse finding was shrinkage of the prostate gland in males. This occurred at dosages of 15 mg/kg/d and higher, which provided systemic exposures greater than or equal to three times the human therapeutic AUC. Prostate shrinkage was not associated with altered reproductive behavior, reproductive function, or semen quality. The mechanism for prostate size reduction in dogs appears to be blockade of the androgen receptors at eplerenone concentrations in excess of those needed to block aldosterone receptors. This hypothesis is supported by *in vitro* data showing that eplerenone can inhibit binding of dihydrotestosterone to dog prostate androgen receptors at concentrations roughly similar to those associated with the *in vivo* effect. Other studies showed lack of effects on the *in vitro* conversion of testo-

sterone to dihydrotestosterone and on the *in vivo* synthesis of testosterone or luteinizing hormone in response to gonadotropin-releasing hormone.

Carcinogenicity and genotoxicity

A 2-year carcinogenesis study of eplerenone in the rat was conducted at dosages of 20, 75, and 250 mg/kg/d administered by gavage. Stimulation of the thyroid gland was the principal finding. Statistically significant increases of benign thyroid follicular cell adenomas occurred in both sexes at 250 mg/kg/d and in males only at 75 mg/kg/d. Systemic exposures that resulted in thyroid adenomas were approximately 2.4 to 13 times higher than the AUC at the 100-mg human dose. There were no treatment-related increases in malignant thyroid tumors or in any other tumor type. As indicated previously, the thyroid stimulation is considered to be secondary to hepatic induction of UDPGT, one form of which is the rate-limiting enzyme for thyroxine clearance, and subsequent compensatory increased TSH. The development of thyroid follicular tumors in rats by this mechanism is common to many drugs and chemicals and is considered to be rodent-specific and not relevant to humans (3,7,23,29).

A carcinogenesis assessment was also conducted in a second rodent species, the genotoxin-sensitive heterozygous P53 knockout mouse (13,16,52), at dosages of 100, 300, and 1000 mg/kg/d. No eplerenone-related tumors developed in these mice.

Eplerenone was not genotoxic in an extensive battery of *in vitro* and *in vivo* assays designed to test its potential mutagenicity and clastogenicity.

Reproductive Toxicity

Reproductive toxicology testing included assessment of fertility and early embryonic development (Segment I) in the rat, embryo-fetal development (Segment II) in the rat and rabbit, and pre and postnatal development in rats (Segment III). Segment I studies showed no adverse effects on the health or fertility of the rats at dosages up to 300 mg/kg/d. At 1000 mg/kg/d, when treated males were bred with untreated females, there was a slight increase in the postimplantation loss of embryos. In rat Segment II studies, there were no compound-related fetal anomalies at any dosage. However, at a dosage of 1000 mg/kg, the dams had reduced feed consumption and body weight gain and the fetal weights also were reduced compared with controls. In a rabbit Segment II study, there were also no compound-related fetal anomalies, but at the maximum dose of 300 mg/kg/d, there was an increase in early resorptions associated with maternal toxicity (decreased body weights and decreased food consumption). A study of pre and postnatal development through F1 generation mating (Segment III) was conducted in rats. At 300 and 1000 mg/kg/d, body weights of the dams were slightly decreased relative to controls. At 1000 mg/kg, body weights also were decreased in the F1 offspring beginning at birth, but there were no effects on the postnatal development or mating performance of the F1 offspring, which were exposed to eplerenone through the mothers' milk.

Safety Pharmacology

A battery of safety pharmacology studies (pharmacology studies performed at dosages above the pharmacologic range) of eplerenone showed no adverse effects. These studies

included cardiovascular assessment in the dog, cardiopulmonary assessments in the guinea pig, neurobehavioral testing in the rat, hepatic effects in the rat, and antigenicity testing in several species.

CLINICAL TRIALS

Phase II and III clinical trials designed to establish the safety, efficacy, and tolerability of eplerenone for the treatment of hypertension and symptomatic heart failure have either been completed or are underway. The Phase II dose-ranging hypertension study demonstrated that eplerenone is a safe, efficacious, well-tolerated antihypertensive using either once-daily (q.d.) or twice-daily (b.i.d.) dosing as compared to placebo and spironolactone (17–19). The Phase II heart failure study demonstrated that eplerenone is an efficacious aldosterone receptor antagonist with a greater safety profile than spironolactone (35). Data from the Phase III trials are not yet available.

Hypertension

The Phase II, multicenter, randomized, double-blind, placebo-controlled, parallel group, dose-ranging, 8-week study compared eplerenone 50, 100, or 400 mg daily as a single dose or in 2 divided doses to spironolactone (50 mg b.i.d.) and placebo in 417 patients with mild to moderate hypertension using cuff and 24-h ambulatory blood pressure monitoring (ABPM) (17–19).

Patients with mild to moderate hypertension (diastolic blood pressure, DBP, ≤ 95 mm Hg and < 114 mm Hg by cuff, and mean 24-h ABPM DBP ≥ 85 mm Hg), were randomized to placebo, eplerenone (either 25, 50, or 200 mg b.i.d.; or 50, 100, or 400 mg q.d.), or spironolactone 50 mg b.i.d. Primary endpoints were mean change from baseline in trough cuff DBP and safety. Secondary endpoints included mean change from baseline in trough cuff SBP, and RAAS hormones (total and active plasma renin, serum aldosterone).

Within the eplerenone-treated groups, adjusted mean changes from baseline at week 8 (final visit) for SBP/DBP were: seated, -4.4 to -15.0 / -4.4 to -8.9 mm Hg; 24-h ABPM, -6.2 to -16.1 / -4.1 to -9.0 mm Hg. Within the spironolactone-treated group, adjusted mean changes from baseline at week 8 for SBP/DBP were: seated, -16.7 / -9.5 mm Hg; 24-h ABPM, -15.8 / -8.7 mm Hg. Consequently, eplerenone caused similar reductions in blood pressure when compared with spironolactone and significant reductions when compared with placebo ($P \leq 0.01$) regardless of assessment method. In addition, trough-to-peak ABPM ratios showed that eplerenone maintains 24-h antihypertensive control with q.d. dosing. Overall, a clear dose-response effect for reductions in SBP and DBP was produced using eplerenone 50 to 400 mg daily.

In addition, eplerenone did not cause the antiandrogenic or progestational side effects, typical of spironolactone, yet there was a dose-related increase in plasma renin (total and active) and serum aldosterone values that indicated that eplerenone was an effective aldosterone receptor antagonist. The incidence of adverse effects was the same as with placebo, and no deaths occurred. Consequently, eplerenone is a safe, efficacious, well-tolerated antihypertensive drug.

Heart Failure

The Phase II, multicenter, randomized, double-blind, placebo- and active-controlled, parallel group, dose-ranging, 12-week study compared eplerenone 25 mg b.i.d. and 25 to 100 mg q.d. with spironolactone 25 mg q.d. and placebo in 321 patients with symptomatic heart failure (35). Randomized patients exhibited stable NYHA class II to IV heart failure, a mean age of 61 years and left ventricular ejection fraction (LVEF) $\leq 40\%$. They were maintained on standard therapy including an ACE inhibitor and a loop diuretic with or without digoxin.

Patients were followed for 12 weeks for determination of changes in neurohormones including brain natriuretic peptide (BNP), urinary aldosterone, plasma renin and testosterone as well as serum potassium, blood pressure, heart rate, and NYHA class. After 12 weeks of treatment, patients received a doubled dose of eplerenone and were followed for an additional 4 weeks to provide safety analysis data.

After 12 weeks, patients on both eplerenone and spironolactone demonstrated a significant decrease in BNP, and an increase in urinary aldosterone and renin in comparison to placebo ($P \leq 0.05$), beginning at a dose of eplerenone 50 mg daily. There was a clinically important increase in the incidence of hyperkalemia (potassium >6.0 mEq/L) with eplerenone 100 mg daily compared with spironolactone (12.0% versus 8.7%). Male patients treated with spironolactone experienced a significant increase in total testosterone compared with eplerenone-treated men ($P \leq 0.02$). Investigators attributed the increase to positive feedback in response to the blockade of androgen receptors. There were no significant changes in NYHA class or body weight in patients treated with eplerenone or spironolactone as compared with placebo. Overall, eplerenone was safe and well tolerated compared with spironolactone and placebo in patients with heart failure maintained on standard therapy including an ACE inhibitor.

Phase III clinical trials are currently underway to assess the safety and efficacy of eplerenone in a broad therapeutic range of patients with mild to moderate hypertension. Phase III endpoint trials are also underway to determine if eplerenone can reduce the mortality associated with symptomatic heart failure. Data are not yet available.

SUMMARY

Eplerenone is a SARA that has been shown to bind avidly to MR, while binding poorly to other steroid receptors. In rodent models, eplerenone provides marked protection against vascular injury in the kidney and heart. Moreover, after MI, eplerenone did not affect myocardial infarct healing, but provided protection against reactive fibrosis. In pre-clinical and clinical pharmacokinetic studies, eplerenone was shown to have good bio-availability with low protein binding, good plasma exposure, and was highly metabolized to inactive metabolites and excreted principally in the bile. Eplerenone was well tolerated in acute and chronic safety pharmacology studies, with the primary findings related to predictable changes in electrolytes or findings that are known to be specific to preclinical species. Safety findings occurred at exaggerated doses that produced exposures several-fold the multiple of the predicted efficacious clinical exposure of hypertensive patients. In phase II clinical trials, eplerenone was well tolerated and had a significant and comparable antihypertensive effect when dosed once or twice a day, and had a peak-to-trough ratio

that indicates 24-h control of blood pressure. In patients with heart failure, eplerenone was safe and well tolerated when given with standard of care.

CONCLUSION

Eplerenone will be the first drug in a new class of SARAs. The ability to effectively block the MR while avoiding limiting side effects promises to be an important advancement in the chronic treatment of life-threatening cardiovascular disorders. Ongoing phase III trials in hypertension and heart failure will soon reveal if the promising pre-clinical and phase II clinical data can be extended to large patient populations and are likely to uncover additional benefits of this unique agent.

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