Renin Inhibitor: An Overview

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Summary

Renin inhibitor, a novel class in the antihypertensive therapy which a prototype drug Aliskerin. This class can be the better of choice and current article covers the history, classification, marketed formulations and pharmacokinetic parameters of various drugs in the class of rennin inhibitors along with the drugs in the clinical trials.

Keywords: Renin, Renin Inhibitors, Aliskiren, Antihypertensive.

Introduction

Drugs that inhibit the renin-angiotensin system, such as angiotensin converting enzyme (ACE) inhibitors and angiotensin receptor antagonists, have proven value for the treatment of hypertension, heart failure and renal disease. They reduce the rates of death, myocardial infarction and stroke in a broad range of patients at high risk, but do not control the blood pressure in all cases. This led to research into inhibiting the renin-angiotensin system at its first step – the production of angiotensin me.[1]

Use of drugs that inhibit the renin-angiotensin system is an effective way to intervene in the pathogenesis of cardiovascular and renal disorders. The idea of blocking the renin system at its origin by inhibition of renin has existed for more than 30 years. Renin inhibition suppresses the generation of the active peptide angiotensin II. The first generation of orally active renin inhibitors were never used clinically because of low bioavailability and weak blood-pressure-lowering activity. At present, aliskiren is the first non-peptide orally active renin inhibitor to progress to phase-III clinical trials. It might become the first renin inhibitor with indications for the treatment of hypertension and cardiovascular and renal disorders. Novel compounds with improved oral bioavailability, specificity, and efficacy are now in preclinical development. Use of drugs that inhibit the renin-angiotensin system is an effective way to intervene in the pathogenesis of cardiovascular and renal disorders.

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HISTORY

The first evidence of the existence of renin was presented over 100 years ago. However, the importance of renin and the renin–angiotensin system in the pathogenesis of cardiovascular disease was only fully realized in the 1970s. It was another 20 years before the first inhibitors of renin were available for clinical research. Here, we describe the discovery and development of aliskiren, an orally active renin inhibitor, which became the first drug in its class to receive regulatory approval. In 2007, it was approved for the treatment of hypertension by the US Food and Drug Administration and the European Medicines Agency. [4]

Aliskiren, is a first-in-class oral renin inhibitor, developed by Novartis in conjunction with the biotech company Speedel. It was approved by the US Food and Drug Administration in 2007. It is an octanamide, is the first known representative of a new class of completely non-peptide, low-molecular weight, orally active transition-state renin inhibitors. Designed through the use of molecular modeling techniques, it is a potent and specific in vitro inhibitor of human renin (IC50 in the low nanomolar range), with a plasma half-life of ≈24 hours. Aliskiren has good water solubility and low lipophilicity and is resistant to biodegradation by peptidases in the intestine, blood circulation, and the liver. It was approved by the United States FDA on 6 March 2007, and for use in Europe on 27 August 2007. Its trade name is Tekturna in the USA, and Rasilez in the UK. [4,5]

While Novartis was developing inhibitors by modification of the peptide-like inhibitors of renin, Hoffman-La Roche started developing renin inhibitors, which were completely different in structure, having a piperidine ring. Screening of the Roche compound libraries led to the identification of rac-2(molecule a) (piperidine structure) which was selective in inhibiting renin over other aspartic proteases. Hoffman-La Roche pursued the development of these compounds until 2001 advancing to pre-clinical stage. Based on the piperidine structure, Pfizer pursued the

task of designing ketopiperazine-based renin inhibitors which have shown greater potential(molecule b). More recently a new series of renin inhibitors based on the ketopiperazine structure was developed by Actelion Pharmaceuticals. These molecules have a 3,9-diazabicyclo[3.3.1] nonene group in place of the ketopiperazine group (molecule c). Another group of chemists from Vitae Pharmaceuticals has developed orally bioavailable alkyl amines based solely on a computational structure-based design (molecule d). [5,6]

Examples

- Aliskiren
- Remikiren
- enalkiren

RENIN INHIBITOR DETAIL

Direct Renin Inhibitor Falls Short In Study Of Heart Attack Patients
Aliskiren did not help proven therapies to prevent changes in heart's shape and function

Atlanta, GA – In patients recovering from a heart attack, adding a medication that directly blocks the action of the hormone renin did not help proven therapies prevent changes in the heart's shape and function, according to research presented today at the American College of Cardiology's 59th annual scientific session. ACC.10 is the premier cardiovascular medical meeting, bringing together cardiologists and cardiovascular specialists to further advances in cardiovascular medicine. Aliskiren Study in Post-MI Patients to Reduce Remodeling (ASPIRE) found that adding aliskiren to the best medical therapy – including an angiotensin-converting-enzyme (ACE) inhibitor or an angiotensin-receptor blocker (ARB) – had no additional beneficial effect on left ventricular remodeling after myocardial infarction (MI), a process that increases the size of the heart and can reduce its ability to pump blood efficiently. In addition, more patients developed high levels of potassium in the blood and low blood pressure. "Morbidity and mortality remains high in patients following heart attack, with a substantial number of patients subsequently developing heart failure," said Scott D. Solomon, M.D., director of noninvasive cardiology at the Brigham and Women's Hospital, Harvard Medical School, Boston. "Reducing harmful ventricular remodeling is one way to improve outcomes in post-heart attack patients. We tested a new way to inhibit the renin-angiotensin system on top of standard therapy in high-risk post-heart attack patients to determine if this therapy would further reduce left ventricular remodeling and thereby minimize the negative outcomes following heart attack. We hoped that this study would generate the information needed to plan a major morbidity and mortality trial. However, our results show that the addition of aliskiren to standard therapy in high-risk post-MI patients does not affect left ventricular size or function. These findings suggest the need for caution when treating post-heart attack patients." Perhaps 25 to 40 percent of patients who survive a heart attack experience a reduction in the heart's pumping ability, or left ventricular dysfunction, as a result of scarring of the heart muscle and left ventricular remodeling. Over time, this process can lead to signs and symptoms of heart failure. The renin-angiotensin-aldosterone system (RAAS) plays an important role in left ventricular remodeling by causing the arteries to constrict and the kidneys to retain fluid and sodium, both of which raise blood pressure and put an extra strain on the heart. Aliskiren inhibits renin, the first enzyme in the RAAS cascade. By comparison, ACE inhibitors block the conversion of angiotensin 1 to its

active form, angiotensin 2, and ARBs block the angiotensin receptor. For the study, researchers recruited 820 patients two to six weeks after a heart attack. All patients had evidence of left ventricular dysfunction; with at least 20 percent of the heart unable to contract because of scarring and an ejection fraction of 45 percent or less. Ejection fraction measures the proportion of the blood in the left ventricle that is pumped into the circulation with each beat. A normal ejection fraction is at least 50 percent; the average ejection fraction in the ASPIRE study was 38 percent. Patients were randomly assigned to receive aliskiren, starting with 75 mg daily and increased to 300 mg daily within two weeks, or a matched placebo. All patients also received the best available medical therapy, including an ACE inhibitor or an ARB. A total of 672 patients had interpretable baseline and follow-up echocardiograms at 36 weeks to evaluate the change in the heart's size and function. Left ventricular end-systolic volume – the volume of the left ventricle when it is contracting and squeezing out blood – was reduced in size by an average of 4.4 mL in the aliskiren group and 3.5 mL in the placebo group, a statistically insignificant difference. Researchers also observed no difference between the two groups in how much the end-diastolic volume—the volume of the left ventricle during relaxation—changed over time, or in the ejection fraction. During follow-up, the combined rates of cardiovascular death, hospitalization for heart failure, recurrent heart attack, stroke and resuscitated sudden death were similar in the two groups. However, in patients receiving aliskiren there was a higher rate of hyperkalemia (potentially dangerous levels of potassium in the blood), more hypotension, (low blood pressure) and more kidney dysfunction, when compared to the placebo group.

"This is the first trial of high-risk, post-heart attack patients treated with the direct renin inhibitor aliskiren," Solomon said. "Our results are consistent with previous studies that showed no benefit, and potentially greater risk of adverse events, when combining two inhibitors of the renin-angiotensin system. Given these results, we are not currently recommending the use of this agent in addition to other inhibitors of the renin-angiotensin system in this specific patient population. However, additional ongoing morbidity and mortality studies with aliskiren are well underway in patients with heart failure and diabetic kidney disease to determine the role for this agent in these populations." The ASPIRE study was funded by Novartis. Dr. Solomon has received research support from Novartis and consults with the company.*r.* [6,7,8]

Oral renin inhibitors

Use of drugs that inhibit the renin-angiotensin system is an effective way to intervene in the pathogenesis of cardiovascular and renal disorders. The idea of blocking the renin system at its origin by inhibition of renin has existed for more than 30 years. Renin inhibition suppresses the generation of the active peptide angiotensin II. The first generation of orally active renin inhibitors were never used clinically because of low bioavailability and weak blood-pressure-lowering activity. At present, aliskiren is the first non-peptide orally active renin inhibitor to progress to phase-III clinical trials. It might become the fi rst renin inhibitor with indications for the treatment of hypertension and cardiovascular and renal disorders. Novel compounds with improved oral bioavailability, specifi city, and effi cacy are now in preclinical development. This Review summarises the development of oral renin inhibitors and their pharmacokinetic and pharmacodynamic properties, with a focus on aliskiren. Inhibition of the renin-angiotensin system is an eff ective way to intervene in the pathogenesis of cardiovascular and renal disorders.1 Renin controls the fi rst rate-limiting step of the system and cleaves angiotensinogen to the inactive decapeptide angiotensin I. The active octapeptide angiotensin II is formed from angiotensin I by the angiotensin-converting enzyme. Angiotensin II acts via type-1 angiotensin II

receptors (AT1) to increase arterial tone, adrenal aldosterone secretion, renal sodium reabsorption, sympathetic neurotransmission, and cellular growth. The renin system can be inhibited at various points β blockers reduce the release of renin from the juxtaglomerular apparatus and lower blood pressure. Inhibitors of angiotensin-converting-enzyme (ACE) reduce the conversion of angiotensin I to angiotensin II. ACE inhibitors also inhibit the inactivation of bradykinin and substance P. These peptides mediate some of the side-effects of ACE inhibitors, such as cough5 and angiooedema. Angiotensin-receptor blockers specifi cally interfere with the interaction of angiotensin II with the AT1 receptor, but do not oppose stimulation of the angiotensin II type-2 receptor. Inhibition of rennin activity blocks the renin system at its very origin. ACE inhibitors, angiotensin-receptor blockers, and renin inhibitors interrupt the normal feedback suppression of renin secretion from the kidneys .The reactive rise in circulating active rennin leads to greater generation of angiotensin I which in turn increases the formation of angiotensin II via pathways dependent or independent of the ACE . Such escape processes do not occur with β blockers . Renin inhibitors do not block renin-like enzymes, such as cathepsin D or tonins, which are present in the vascular wall and which release angiotensin I from angiotensinogen. Renin has a unique specifi city for its only known physiological substrate, angiotensinogen. By 1957, Skeggs11 had already outlined the potential benefits of specific inhibition of the renin system by diminishing renin activity without interference with other metabolic path ways. Development of oral renin inhibitors. The sequence of renin differs between species, so that preclinical studies of renin inhibitors must be done in primates, such as marmosets, or in rat models transgenic for human renin and angiotensinogen.12 The earliest attempts to block the renin system relied on antibodies raised against renin. Immunological inhibition of renin reduced blood pressure in volume depleted normotensive marmosets15 and provided the proof of concept of renin inhibition. The first synthetic renin inhibitor was pepstatin. First-generation renin inhibitors were peptide analogues of the prosegment of renin17 or substrate analogues of the amino-terminal sequence of angiotensinogen containing the renin cleavage site.18–20 They had to be given parenterally, but were effective at inhibiting rennin activity and reducing blood pressure in animals19 and in people.21 Further chemical modification led to the development of compounds, such as CGP29287, that had greater stability and longer duration of action. CGP29287 was the first rennin inhibitor to show activity when given orally; it was orally active in marmosets at high doses.22 In the second half of the 1980s, several drug companies developed rennin inhibitors that had a molecular weight of a tetrapep. [8,9]

Marketed Renin Inhibitor

Aliskire(2(*S*),4(*S*),5(*S*),7(*S*)-*N*-(2-carbamoyl-2-methylpropyl)-5-amino-4-hydroxy-2,7 diisopropyl-8-[4-methoxy-3-(3-methoxypropoxy)phenyl]- octanamid hemifumarate) is the first in a new class of orally active, nonpeptide direct renin inhibitors developed for the treatment of hypertension. The absorption, distribution, metabolism, and excretion of [14C]aliskiren were investigated in four healthy male subjects after administration of a single 300-mg oral dose in an aqueous solution. Plasma radioactivity and aliskiren concentration measurements and complete urine and feces collections were made for 168 h postdose. Peak plasma levels of aliskiren (*C*max) were achieved between 2 and 5 h postdose. Unchanged aliskiren represented the principal circulating species in plasma, accounting for 81% of total plasma radioactivity (AUC0–_), and indicating very low exposure to metabolites. Terminal half-lives for radioactivity and aliskiren in plasma were 49 h and 44 h, respectively. Dose recovery over 168 h was nearly complete (91.5% of dose); excretion occurred almost completely via the fecal route (90.9%),

with only 0.6% recovered in the urine. Unabsorbed drug accounted for a large dose proportion recovered in feces in unchanged form. Based on results from this and from previous studies, the absorbed fraction of aliskiren can be estimated to approximately 5% of dose. The absorbed dose was partly eliminated unchanged via the hepatobiliary route. Oxidized metabolites in excreta accounted for at least 1.3% of the radioactive dose. The major metabolic pathways for aliskiren were *O*-demethylation at the phenyl-propoxy side chain or 3-methoxy-propoxy group, with further oxidation to the carboxylic acid derivative. Hypertension is a major risk factor for cardiovascular and kidney diseases, and affects more than 25% of adults worldwide. Despite the known risks associated with hypertension and the availability of a range of antihypertensive drug therapies, the majority of patients with hypertension do not have their blood pressure controlled to recommended target levels (_140/90 mm Hg for most patients). Indeed, data from the National Health and Nutrition Examination Surveys for 1999 to 2002 showed that blood pressure was uncontrolled in more than 70% of patients with hypertension in the United States (Centers for Disease Control and Prevention, 2005). The renin system plays a key role in the physiological regulation of blood pressure and intravascular volume through the actions of the peptide angiotensin II. Excessive renin system activity may lead to hypertension and associated target organ damage Drugs that inhibit the renin system, such as angiotensin converting enzyme inhibitors and angiotensin receptor blockers, have proven to be highly successful treatments for hypertension and related cardiovascular diseases However, all currently available agents that inhibit the renin system stimulate compensatory renin release from the kidney, which results in an increase in plasma renin activity that may ultimately lead to increased levels of angiotensin II., Therefore, targeting the renin system at its point of activation by directly inhibiting renin activity has long been proposed as the optimal means of suppressing the renin system However, previous efforts to develop clinically effective direct rennin inhibitors have been thwarted by the low potency and/or poor pharmacokinetic profiles of peptide-like compounds Previous generation renin inhibitors have exhibited an oral bioavailability of around 1%, because of low intestinal absorption and/or considerable hepatic first-pass metabolism Aliskiren (2(*S*),4(*S*),5(*S*),7(*S*)-*N*-(2-carbamoyl-2-methylpropyl)-5-amino- 4-hydroxy-2,7-diisopropyl-8-[4-methoxy-3-(3-methoxypropoxy)phenyl]- octanamid hemifumarate) is the first in a new class of orally effective, nonpeptide direct renin inhibitors developed for the treatment of This study was supported by Novartis Pharma AG, Basel, Switzerland.

Article, publication date, and citation information can be found at Crystallographic structure analysis of subsequent inhibitors revealed a hitherto uncharacterized nonsubstrate subpocket within the human rennin. This allowed the addition of further substituents to fill this subpocket and thus increase affinity for the enzyme, leading to the synthesis of aliskiren, a potent (in vitro IC50 0.6 nM) and highly specific inhibitor of human renin Pharmacokinetic studies in healthy volunteers have demonstrated that aliskiren is rapidly absorbed (*t*max 1–3 h) and exhibits a long plasma half-life (*t*1⁄2 30–40 h) (Vaidyanathan et al., 2006a,b) suitable for oncedaily dosing. Aliskiren demonstrates dose-proportional pharmacokinetics at doses of up to 600 mg once daily in healthy volunteers . Clinical trials have shown that once-daily treatment with aliskiren lowers blood pressure at least as effectively as angiotensin receptor blockers and angiotensin-converting enzyme inhibitors in patients with hypertension. Studies investigating the disposition of oral doses of [14C]aliskiren in rats and marmosets indicated that excretion of an oral dose occurred almost exclusively in the feces, mainly as unchanged aliskiren; a small proportion of the absorbed dose was excreted in the form of oxidized metabolites, probably

derived from oxidation by CYP3A4. However, no interaction of aliskiren with cytochrome P450 (P450) isoenzymes was found in human liver microsomes in vitro suggesting a low potential for clinically significant drug interactions of aliskiren. Indeed, no clinically relevant pharmacokinetic interactions have been observed between aliskiren and the P450 substrates celecoxib, digoxin, lovastatin, or warfarin, or the P450 inhibitor cimetidine, in healthy volunteers. Animal studies indicate that aliskiren is a substrate for the efflux transporter Pglycoprotein, which may play a role in the hepatobiliary/intestinal excretion of the drug; however, the lack of pharmacokinetic interaction between aliskiren and the P-glycoprotein substrate digoxin indicates that aliskiren does not inhibit P-glycoprotein activity . The aim of the present study was to characterize the absorption, distribution, metabolism, and excretion of a single 300-mg oral dose of [14C]aliskiren in healthy male subjects. [10,11,12]

Materials And Methods

Clinical Study and Subjects. The study was performed at Swiss Pharma Contract (SPC) Ltd . Four healthy, nonsmoking male subjects, aged 26 to 47 years, with normal medical history, vital signs (body temperature, blood pressure, and heart rate), 12-lead electrocardiograph, and laboratory tests participated in this open-label study. All patients had body weight within _20% of normal for their height and frame size according to Metropolitan Life Insurance Tables. [13]

Exclusion criteria included exposure to radiation greater than 0.2 mSv in the 12 months before the start of the study; use of any prescription drug, overthe- counter medication (except paracetamol), grapefruit juice, St John's wort, and/or herbal remedies in the 2 weeks before the study; and a history of any condition known to interfere with the absorption, distribution, metabolism, and excretion of drugs. The study was conducted in accordance with Good Clinical Practice guidelines and the Declaration of Helsinki (1964 and subsequent revisions), and all patients gave written informed consent before participation. The subjects were exposed to a radiation dose _1 mSv, which was calculated according to the guidelines of the International Commission on Radiological Protection and Swiss regulations. The protocol and the dosimetry calculation were approved by the local ethics committee and by the Swiss Federal Health Authority Radiation Protection Department. Study Medication. Aliskiren was specifically labeled with 14C in the 2-methyl groups; this position is metabolically stable. The radioactive label had a specific activity of 9.27 kBq/mg (0.25 _Ci/mg) as 300 mg free base and 55.56 kBq/ml in 50 ml of drink solution, and a radiochemical purity of _99%. The established solid dosage form of aliskiren could not be manufactured with 14C-radiolabeled drug substance because of radiochemical instability. The radiolabeled drug was stable in aqueous solution, frozen at _20°C. Subjects therefore received a single 300-mg oral dose of [14C]aliskiren mg of hemifumarate salt containing a mean dose of radioactivity of 2.8 MBq (75 _Ci), in the form of an oral solution (in 50 ml of water). After dose administration, the solution container was rinsed twice with 50 ml of water, which was also swallowed by the subjects. Study Protocol. After a screening period of up to 21 days, eligible subjects reported to the study center at least 16 h before dosing for baseline safety evaluations and were domiciled in the study center for the 168-h postdose observation period. Safety and pharmacokinetic assessments were performed for up to 336 h postdose. A single 300-mg oral dose of aliskiren was administered to all subjects in the morning, after an overnight fast of at least 10 h. Blood samples were collected by direct venipuncture or an indwelling catheter into heparinized tubes predose and at 0.25, 0.5, 0.75, 1, 1.25, 1.5, 2, 3, 4, 5, 6, 8, 10, 12, 16, 24, 48, 72, 96, 120, 144, and 168 h postdose. Three aliquots

of 0.3 ml each were taken from each sample and frozen immediately at __20°C for subsequent radiometry. Plasma was prepared from the remaining blood by centrifugation at 4°C for 10 min at 2000*g*. Urine was collected predose and at 0 to 6, 6 to 12, 12 to 24, 24 to 48, 48 to 72, 72 to 96, 96 to 120, 120 to 144, and 144 to 168 h postdose, in a total of 10 fractions. Fecal samples were collected predose and thereafter up to 168 h postdose; each portion was diluted with 2 to 3 volumes of water and homogenized. Blood plasma, urine, and feces were stored at __20°C until required for analysis. Blood samples collected on days 10, 12, and 15 were not analyzed for radioactivity because the terminal elimination phase for aliskiren could be characterized sufficiently with the samples collected in the time period 48 to 144 h. Throughout the study, subjects were not permitted to perform strenuous physical exercise (for 7 days before dosing until after the end of study evaluation) or to take alcohol (for 72 h before dosing until the end of the study) or citrus fruit or fruit juices (for 48 h before dosing throughout the domiciled period). Intake of xanthine-containing food or beverages was also not permitted from 48 h before dosing until 48 h postdosing. Consumption of other foods that might lead to interactions with study drug or lead to technical problems in the analysis of excreta was also not permitted during the domiciled period. Analysis of Unchanged Aliskiren. *Plasma sample preparation.* Plasma samples were cleaned by automated solid-phase extraction using a 96-well plate and Oasis MCX 10-mg extraction cartridges (Waters Corporation, Milford, MA) on a Multiprobe II After the conditioning steps [500 _l of methanol/water (90:10 v/v) containing 1% acetic acid, 500 _l of 1% acetic acid in water], 600 _l of acidified sample was transferred to the well. The sample was washed twice with acetic acid (1% in water), and once with methanol/acetonitrile (40:60 v/v). After the elution step [300 _l of methanol/water (90:10, v/v) containing Structure of [14C]aliskiren. [14,15]

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The extract was partially evaporated (concentration by approximately 2-fold) and then diluted with 150 _l of 1% acetic acid in water. *Urine sample preparation.* Urine samples were cleaned by automated solidphase extraction using a 96-well collection plate and Oasis MCX 10-mg extraction cartridges, on a Multiprobe II. After the conditioning steps (200 _l of methanol, then 200 _l of pH 12 buffer), 200 _l of alkalinized sample wastransferred to the well. The sample was washed with 400 _l of methanol/water (25:75, v/v). After the elution step [300 _l of acetonitrile/water (90:10 v/v) containing 1% acetic acid], the extract was partially evaporated (concentration by approximately 2-fold) and then diluted with 200 _l of 1% acetic acid. [16]

HPLC-MS/MS analysis. HPLC was performed using a MetaSil Basic 5-_m column (50 _ 2.0 mm; column temperature 40°C, flow rate 0.25 ml, injection volume 10 _l; Metachem, Palo Alto, CA) with gradient elution from 10 mM aqueous ammonium acetate/acetonitrile (75:25 v/v) to 10 mM aqueous ammonium aetate/acetonitrile (40:60 v/v) over 0.4 min. An API 3000 (Applied Biosystems, Foster City, CA) was used for mass spectrometry. The general settings used were selected reaction monitoring, positive ion mode, and electrospray ionization interface; temperature 500°C, mass resolution 0.7 atomic mass unit, scan time 0.50 s. The lower limit of detection for the HPLC-MS/MS assay was 0.5 ng/ml for plasma and 5 ng/ml for urine. A derivative of aliskiren (gem-dimethyl *d*6-aliskiren) was used as an internal standard. Total Radioactivity Measurement. Total 14C radioactivity in blood and plasma was measured at

Novartis Pharma AG using liquid scintillation counting (LSC). Blood and plasma samples (triplicates of 1300 _l each, weighed) were counted after solubilization in Biolute *S*-isopropanol and LSC used RiaLuma . LSC was performed using a Tri-Carb 3170 TR/SL liquid scintillation counter ("low-level counter"; PerkinElmer Life and Analytical Sciences). Counting was performed for 60 or 180 min per sample in low level counting mode. Total 14C radioactivity in urine and feces was measured at RCC Ltd. using LSC with a typical counting time of 10 min. Fecal samples (quadruplicates of 400 mg each, weighed) were counted after homogenization in 2 to 3 volumes LSC used Irga-Safe Plus

Urine samples (duplicates of 1 ml each) were measured directly with scintillation cocktail (Irga-Safe Plus). LSC was performed using a Tri-Carb 2500 TR, 2550TR/LL, or 2900TR liquid scintillation counter (Packard Biosciences). Quench correction was performed by the external standard method. The background for blood and plasma was determined and subtracted from the measurements of study samples. The limit of quantification (LOQ) of LSC was determined as described previously (Jost et al., 2006) and was defined as the

minimal number of sample disintegrations that are statistically significant above background and that show a relative statistical uncertainty equal to or smaller than 20%. Thus, the LOQ was 17 ng-Eq/ml (2.8 dpm) for blood (counting time 60 min), 11.4 ng-Eq/ml (1.8 dpm) for plasma (counting time 180 min), and approximately 0.01% of dose for urine and feces. Radioactivity levels in plasma samples collected at 16 and 144 h postdose were below the LOQ of LSC and were therefore analyzed using accelerator mass spectrometry (AMS) by Xceleron Ltd. Samples were thawed and centrifuged at 4000*g* for 5 min at 10°C; 60-_l aliquots of plasma were then dried under a vacuum with copper oxide, combusted (at 900°C for 2 h), reduced to graphite, and analyzed using AMS, which separates the carbon isotopes and determines specifically the 14C isotope. Biologic sample preparation for metabolite profile analysis. For the following sample preparation processes, radioactivity was traced by quantitative radiometric measurements of aliquots using a Tri-Carb 2500TR liquid scintillation counter as described previously Plasma. A plasma sample of 2 ml was mixed with 2 ml ice-cold acetonitrile. After 30 min on ice, the sample was centrifuged (17,500 g, 15 min) and the supernatant was withdrawn. The extract was then concentrated in a rotary evaporator to a volume of 0.7 to 1.1 ml. An aliquot was taken for determination of total radioactivity by LSC; the rest of the sample (0.6–1 ml) was analyzed by HPLC to obtain the metabolite profile. The overall recovery from sample processing and analysis was 88%. Urine. Individual urine samples were centrifuged and 1 ml supernatant directly injected for HPLC analysis. The recovery from sample processing and analysis was complete. Feces. From each subject, the two samples of feces homogenate that contained the most of the applied radioactivity were pooled. Thus more than 98% of the radioactivity excreted with bile/feces was covered. Approximately 2 g of pooled feces homogenate were mixed with 2 ml water and 4 ml acetonitrile and shaken for 30 min. After centrifugation at 10,000 g for 15 min, the supernatant was withdrawn and a 200 _l sample was directly injected for HPLC analysis. The overall recovery from sample processing and analysis was 90%. Metabolite analysis by HPLC-radiometry. Samples of plasma, urine and feces extract were chromatographed by reversed-phase HPLC with subsequent radioactivity detection. HPLC analysis was performed on an Agilent 1100 HPLC chromatographic system, incorporating a capillary pump G1376A, a degasser G1379A, a thermostat sample holder G1329A (set at 15°C), a column thermostat G1316A (set at 40°C), and a diode array multiwavelength UV detector G1315B (set at 235 nm). Chromatographic separation was performed on a LiChrospher 100-5 RP-18 ec column (5 _m,

250 _ 2 mm; Macherey-Nagel, Du¨ren, Germany) protected by a guard filled with the same material. Gradient elution using mobile phase solvent A (50 mM ammonium acetate adjusted to pH 6.0 with acetic acid) and solvent B (acetonitrile) was applied at a flow rate of 0.25 ml/min as follows: 0 to 35 min, 10 to 30% solvent B; 35 to 45 min, 30% solvent B; 45 to 50 min, 30 to 40% solvent B; 50 to 65 min, 40 to 90% solvent B; 65 to 70 min, 90% solvent B. Samples of 200 to 1000 _l were injected via a 1-ml loop into the HPLC system. Radioactivity was detected offline by collecting the eluate in 0.25-min fractions into three 96-well Deepwell LumaPlates (PerkinElmer Life and Analytical Sciences) by means of an Agilent 1100 fraction collector (Agilent Technologies). After solvent evaporation in a SpeedVac Plus SC210A vacuum centrifuge (Thermo Fisher Scientific, Waltham, MA), radioactivity was determined (counting time 20 min, three times) on a TopCount NXT microplate scintillation and luminescence counter (Packard Biosciences). Metabolite Characterization by HPLC-MS. Selected pooled extracts of urine and feces from individual subjects were analyzed directly by LC-MS with simultaneous radioactivity detection. For confirmation of proposed structures of metabolites of aliskiren, the retention times in the radiochromatograms and mass spectral data obtained in the current study were compared with those obtained for reference compounds and samples from a parallel study in rabbits.[17,18]

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Early apparent half-lives for elimination from plasma (by noncompartmental analysis) were 1.8 h for radioactivity and 2.1 h for aliskiren (difference not significant). Terminal half-lives of radioactivity and aliskiren were 49 h and 44 h, respectively. Approximately 81% of total plasma radioactivity (AUC0–_; 86% for AUC0–10h) was accounted for by unchanged aliskiren, indicating very low exposure to metabolites. Radioactivity in blood was detected up to 4 to 12 h after dosing and was subsequently below the LOQ. The mean ratio of AUC0–10h blood/plasma was 0.61, indicating that radioactivity was largely present in plasma. Excretion and Mass Balance in Urine and Feces. Radioactivity

was excreted almost The majority of fecal excretion of radioactivity (approximately completely via the biliary/fecal route, with only 0.6% of the radioactive dose recovered in urine g). 80% of dose) occurred within 72 h of dosing. Total excretion (mass balance) over the 168-h collection period was 91.5 _ 4.5% of dose, with moderate interindividual variability (range 85–95%). Unchanged aliskiren accounted for 0.4% of dose in urine (approximately 70% of the recovered radioactivity) and for 77.5% of dose in feces (probably _85% of radioactivity); overall, the sum of oxidized metabolites in excreta amounted to approximately 1.4% of the radioactive dose. Metabolism of Aliskiren. *Plasma.* Metabolite patterns in plasma were determined only at *t*max because of the low levels of radioactivity in plasma. At *t*max, unchanged aliskiren accounted for most of the

radioactivity (Fig. 4a). In addition, minor proportions of metabolites M2 (carboxylic acid, oxidized side chain; _1% of aliskiren *C*max) and M3 (alcohol, *O*-demethylated; 1–5% of aliskiren *C*max), and trace levels of M1 (phenol, *O*-demethylated) were detected. These data are semiquantitative because of incomplete 14C extraction recovery (88%). AUC fractions represented by these metabolites in plasma could not be determined accurately because of the low radioactivity at time points after *t*max. *Urine.* Urine samples containing sufficient radioactivity were analyzed for metabolic patterns; thus, one to four urine samples per volunteer

were measured and the sum of the urinary metabolites was calculated. Unchanged [14C]aliskiren accounted for the major part of radioactivity (approximately 70%) in all analyzed urine samples). Using a sensitive, validated HPLC-MS/MS assay for aliskiren, the amount of unchanged aliskiren excreted in urine was determined to be 0.4% of dose. In addition, trace amounts of the metabolites M2, M3, M4 (phenol, *O*-dealkylated), and M6 (*O*-glucuronide conjugate of M4) were detected in urine The unlabeled metabolite M9 (lactone) was also detected by LC-MS. Because of low radioactivity levels, only early urine fractions could be analyzed and the results extrapolated to total amounts excreted in 7 days; in total, M3 amounted to _0.1% of the dose and all other metabolites to trace amounts . *Feces.* The major proportion of the administered radioactive dose was excreted with the feces. For metabolite analysis, a single feces pool was prepared for each volunteer containing at least 78% of the applied radioactivity dose, and by solvent extraction, 90% of the radioactivity was extracted for HPLC analysis. No major differences in fecal metabolite pattern were observed between individual subjects. Unchanged [14C]aliskiren was the predominant compound in the feces; metabolites M2 and M3 were found typically in amounts of 0.7 to 1.2% of the dose In addition, traces of M1 (0.1%) and other peaks were detected LC-MS also detected M4 and the unlabeled metabolite M9 in feces extracts. Feces extracts contained an additional distinct peak close to the aliskiren peak, designated P62, which accounted for approximately 1% of the dose. LC-MS runs under chromatographic conditions identified three separate peaks within P62, corresponding to metabolites M12 (*N*-acetylated), and M13 and M14 (structural isomers containing an additional C3H4O2 moiety in the central part of the molecule). The fact that P62 was only observed in feces extracts suggested that the components of P62 were not systemic metabolites but were formed in gut or feces. This hypothesis was supported by the observation that 14C-plasma concentrations in subject 5101 were distinctly lower than those in the other three volunteers, but the feces extract contained the same proportion of P62 (i.e., 1% of dose) as the other subjects. Metabolite Structure Elucidation. The chemical structures of the metabolites were elucidated essentially based on LC-MS data although in some cases, for complete elucidation, analysis by 1H NMR was required. However, 1H NMR analysis of the human samples was not feasible because of low metabolite concentrations. Therefore, 1H NMR analysis was performed with urinary metabolites, which had been obtained from a parallel rabbit study and which, based on LC-MS data, were identical with the respective human metabolites. The combined data provided unambiguous metabolite identification.

The mass spectrum of the parent compound aliskiren and its proposed interpretation are provided in Fig. 5. Major signals observed were the protonated intact molecule M _ H_ (*m*/*z* 552) and four key fragments (*m*/*z* 436, 209, 137, and 117;). These ions or the mass difference between them can be related to several substructures of the molecule The fragment ions *m*/*z* 436 and *m*/*z* 117 were formed after cleavage of the central amide bond, whereas the fragment ions *m*/*z* 209 and *m*/*z* 137 represent substructures of the 5_ 4.03. [19]

Absorption, Metabolism And Excretion of Aliskiren 1425

Radioactivity was detectable using conventional LSC for up to 12 h. At later time points, analysis required the highly sensitive AMS technique. Single samples were analyzed both with LSC and AMS, with AMS giving 10 to 20% higher values. Therefore, aliskiren accounted for approximately 86% of the plasma radioactivity AUC0– 10h, versus 81% of radioactivity AUC0– _. The difference between LSC and AMS was within common analytical accuracy ranges and thus was not significant. Since AMS has been validated as a quantitative method for 14C

radioactivity (Garner et al., 2000), no systematic method cross-check was performed. The low levels of metabolites of aliskiren in the plasma, urine, and feces suggest a minor role for metabolism in the elimination of aliskiren, but the observed metabolite profile indicates that oxidative processes represent the major pathway for the proportion of aliskiren. Metabolism of aliskiren in humans. a, condensed scheme of metabolism; b, detailed metabolism pathways and metabolite structures; c, proposed derivatives of aliskiren presumably formed in intestine. Dotted arrows indicate potential alternative pathways leading to formation of metabolites M2 and M4. [16,20]

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The two major metabolites, the oxidized derivatives M3 (*O*-demethylated alcohol derivative) and M2 (carboxylic acid derivative) accounted for approximately 3% and 1%, respectively, of the radioactivity in the plasma (at *t*max). An additional oxidized metabolite, M1, was also detected in plasma, and M1 to M3 plus a further oxidized metabolite M4 and traces of its glucuronic acid conjugate (M6), and an unlabeled hydrolysis product (lactone derivative M9) were observed in the urine. With the exception of M6, all of these metabolites were also detected in the feces. Further phase II

conjugation was only observed for the oxidized metabolite M4 (by glucuronic acid conjugation to M6), and there was no evidence for direct glucuronic acid conjugation of aliskiren. The terminal metabolites M1 to M4 accounted for 1.4% of the excreted dose and were all formed by oxidation at the side chain by *O*-demethylation, *O*-dealkylation, and/or alcohol oxidation, probably by CYP3A4 (Novartis, data on file). It is not known whether any aliskiren metabolites exhibit pharmacological activity. However, the very low concentration levels of metabolites as compared with unchanged aliskiren suggest that the metabolites are unlikely to contribute to the biological activity of aliskiren. The trace metabolites M12 *N*-acetyl derivative), and M13 and M14 (which could be characterized only partially) were found only in the feces (in peak P62). Taken together with the observation that the proportions of these metabolites found in the feces were similar in all four subjects (despite notably lower 14C-plasma concentrations in one subject), it seems likely that M12, M13, and M14 are a fecal artifact produced from unabsorbed aliskiren, probably by the intestinal microflora. Indeed, acetylation (which would produce M12) is a metabolic pathway that is known to occur under the anaerobic conditions of the gut (Goldin, 1990). Aliskiren undergoes oxidative metabolism by P450 isoenzymes to a low degree. Aliskiren is not an inhibitor of P450 activity and is unlikely to exhibit pharmacokinetic interactions with drugs that are P450 isoenzyme substrates. An in vitro study showed no notable effects of aliskiren at a concentration of 20 _M (approximately 5-fold higher than the mean *C*max of aliskiren observed in the present study) on the activity of CYP1A2, CYP2C8, CYP2C9, CYP2C19, CYP2D6, effect on aliskiren pharmacokinetics CYP2E1, or CYP3A4. Moreover, studies in healthy volunteers have demonstrated no drug interactions between aliskiren and celecoxib, cimetidine, digoxin, lovastatin or warfarin, all of which are known to interact with P450 isoenzymes., Apart from detailed investigation of pathways for metabolism, the ajor objectives of a human ADME study are assessment of the xtent of absorption and identification of the key elimination processes. The minimal extent of absorption after oral dosing can be estimated as the radioactivity dose proportion excreted in urine, plus the dose proportion excreted in the form of metabolites in feces. However, in the present case, this is not adequate since renal excretion and metabolism are minor, and biliary elimination of unchanged drug is neglected. Therefore, other available data on aliskiren should be considered. 1) In an absolute

bioavailability study in humans based on plasma AUC, the oral bioavailability of aliskiren was determined to be 2.6%. 2) In the same study, the renal excretion of unchanged aliskiren after an intravenous dose of 20 mg was 7.5% of dose. Thus, elimination occurred predominantly via nonrenal processes (ratio of nonrenal/renal approximately 12), including transport with bile and possibly through gut wall, and/or metabolism. 3) In ADME studies in rats and marmosets with oral and intravenous dosing (Novartis, data on file, biliary/fecal dose elimination was predominant; e.g., up to 90% and 78% of intravenous doses were recovered in the feces of rat and marmoset, respectively, largely in the form of unchanged aliskiren. Furthermore, aliskiren has been found to be a substrate for P-glycoprotein, thus intestinal P-glycoprotein might contribute to elimination. On the basis of the absolute bioavailability study, the oral absorption in humans would be at least 2.6% of dose. In the present oral human ADME study, the renal excretion of aliskiren, determined using a sensitive method, was 0.4% of dose, approximately 20 times less than after an intravenous dose. Combined with the results of the intravenous study, an extent of absorption of approximately 5% can be estimated. In the present human ADME study, metabolites accounted for 0.2% of dose in urine. The amount of metabolites formed after absorption and excreted in feces (excluding the fecal metabolite P62, which appears to be formed from unabsorbed aliskiren in the intestine) appears to be at least 1.3% of dose. With various unidentified trace peaks in the fecal metabolite pattern (near detection limit), the total amount of metabolites may have been in the range 1.5 to 3%. Thus, only part of the absorbed aliskiren was eliminated through metabolism. A similar or larger dose fraction, recovered in the feces in unchanged form, must have been due to aliskiren elimination via the hepatobiliary route, and thus, hepatobiliary elimination is concluded to be a main elimination process. Nevertheless, it should be noted that the bulk of the dose excreted in feces is due to unabsorbed drug. Consistent with our findings regarding the elimination of absorbed aliskiren, the pharmacokinetics of aliskiren are not significantly altered by renal impairment (Vaidyanathan et al., 2007a). No significant was found in patients with impaired hepatic function; thus, no dosage adjustment for aliskiren is required (Vaidyanathan et al., 2007b). In the present study, the pharmacokinetics of 14C radioactivity and aliskiren showed large interindividual variability. Indeed, one subject (5101) exhibited a considerably lower exposure to aliskiren than did the other three subjects. The reason is unknown. High variability in aliskiren pharmacokinetic parameters has also been described in clinical studies with solid drug administration (Vaidyanathan et al., 2006a). Since aliskiren is a substrate for P-glycoprotein, interindividual variations in intestinal P-glycoprotein expression might contribute to the observed variability in pharmacokinetics In summary, aliskiren is absorbed to a low extent after an oral dose. Excretion of aliskiren is nearly complete within 168 h, with the majority of an oral dose of aliskiren excreted unchanged in the feces. drug represented the principal circulating species in plasma. Absorbed drug appears to be eliminated via the hepatobiliary route and, to some degree, through oxidative metabolism. [21,22]

Combination Of The Drug

Rasilez is also effective in combination with either a diuretic, a calcium channel blocker (CCB) or an angiotensin receptor blocker

— Updated European Society of Hypertension (ESH) guidelines recognize the benefits of Rasilez® (aliskiren) for the treatment of high blood pressure. Rasilez, a first-in-class direct renin inhibitor (DRI), works at the point of activation of the renin angiotensin aldosterone system (RAAS), directly inhibiting the activity of renin, an enzyme that triggers a process that may lead to high blood pressure and organ damage2,3. The updated European Guidelines on Hypertension

Management, appraised by an ESH task force, recognize that Rasilez effectively lowers high blood pressure in patients when given in monotherapy at a single daily dose, and is also effective when used in combination with either a thiazide diuretic, a calcium antagonist or an angiotensin receptor antagonist. In addition, the guidelines acknowledge that Rasilez has substantially increased its database within the last two years, including data indicating the drug's effects on two indicators of heart failure severity and kidney disease; B-type natriuretic peptide (BNP) and urinary albumin:creatinine ratio (UACR)1,2. The guidelines recognize Rasilez's ability to reduce BNP levels on top of standard therapy in patients with mild stable heart failure and also acknowledge Rasilez's effects on reducing UACR in patients with hypertension, type 2 diabetes mellitus, and nephropathy, on top of standard care1,2.
"There is a serious unmet need in the treatment of high blood pressure and we are very pleased the updated ESH guidelines recognize the benefits of Rasilez as an effective treatment option," said Trevor Mundel, MD, Global Head of Development at Novartis Pharma AG. "An extensive and ongoing cardio-renal outcomes program - ASPIRE HIGHER - will continue to explore Rasilez's long-term benefits and potential to protect against subclinical organ damage beyond existing antihypertensive therapies." The heart and kidney protection potential of Rasilez, in addition to its blood pressure lowering ability, is currently being investigated further in the landmark ASPIRE HIGHER program, the largest ongoing cardio-renal outcomes program worldwide involving more than 35,000 patients in 14 trials. Findings from four of the 14 studies in the ASPIRE HIGHER program, the AVOID, ALOFT, ALLAY and AGELESS studies, have already been reported to date showing that treatment with Rasilez has the potential for cardio-renal protection4-7. About Rasilez/Tekturna Rasilez, known as Tekturna in the US, is the only drug that works by directly targeting renin to decrease the activity of the RAAS2. Renin is an enzyme produced by the kidneys that starts a process that narrows blood vessels and, when inappropriately activated, may lead to high blood pressure. Rasilez reduces plasma renin activity (PRA) and helps blood vessels relax and widen so blood pressure is lowered. [22,23]

Rasilez/Tekturna is approved in over 70 countries. Tekturna was approved in the US in March 2007 and in the European Union in August 2007 under the trade name Rasilez. In July 2009, Rasilez also received approval in Japan. Tekturna HCT, the first single-pill combination involving Tekturna, was approved in the US in January 2008 for second-line treatment of high blood pressure, and more recently for first-line use. The single-pill combination Rasilez HCT was approved in the European Union in January 2009. In September 2009, Valturna, a single-pill combination of Tekturna and Diovan (valsartan), was approved in the US. Other single-pill combinations with Rasilez are currently in development including a single-pill combination with amlodipine. The core of the Novartis portfolio is its cardiovascular and metabolic medications for the treatment of high blood pressure and diabetes. These include Diovan® (valsartan), the number one selling blood pressure medication worldwide8; Exforge® (valsartan/ amlodipine), a single pill combining two leading medicines for high blood pressure; Exforge HCT® (amlodipine/valsartan/HCT); and Rasilez® (aliskiren), the first and only approved direct renin inhibitor, and two single pill combinations of Rasilez, Rasilez HCT (aliskiren/HCT) and Valturna (aliskiren/valsartan). For the treatment of type 2 diabetes, these include Galvus® (vildagliptin, a DPP-4 inhibitor) and Eucreas® (vildagliptin and metformin). [24]

Disclaimer

The foregoing release contains forward-looking statements that can be identified by terminology such as “will,” “potential,” “may,” or similar expressions, or by express or implied discussions regarding potential new indications or labeling for Rasilez/Tekturna or regarding potential future revenues from Rasilez/Tekturna. You should not place undue reliance on these statements. Such forward-looking statements reflect the current views of management regarding future events, and involve known and unknown risks, uncertainties and other factors that may cause actual results with Rasilez/Tekturna to be materially different from any future results, performance or achievements expressed or implied by such statements. There can be no guarantee that Rasilez/Tekturna will be submitted or approved for any additional indications or labeling in any market. Nor can there be any guarantee that Rasilez/Tekturna will achieve any particular levels of revenue in the future. In particular, management’s expectations regarding Rasilez/Tekturna could be affected by, among other things, unexpected clinical trial results, including unexpected new clinical data and unexpected additional analysis of existing clinical data; unexpected regulatory actions or delays or government regulation generally; the company’s ability to obtain or maintain patent or other proprietary intellectual property protection; competition in general; government, industry and general public pricing pressures; the impact that the foregoing factors could have on the values attributed to the Novartis Group's assets and liabilities as recorded in the Group's consolidated balance sheet, and other risks and factors referred to in Novartis AG’s current Form 20-F on file with the US Securities and Exchange Commission. Should one or more of these risks or uncertainties materialize, or should underlying assumptions prove incorrect, actual results may vary materially from those anticipated, believed, estimated or expected. Novartis is providing the information in this press release as of this date and does not undertake any obligation to update any forward-looking statements contained in this press release as a result of new information, future events or otherwise. About Novartis Novartis provides healthcare solutions that address the evolving needs of patients and societies. Focused solely on healthcare, Novartis offers a diversified portfolio to best meet these needs: innovative medicines, cost-saving generic pharmaceuticals, preventive vaccines, diagnostic tools and consumer health products. Novartis is the only company with leading positions in each of these areas. In 2008, the Group’s continuing operations achieved net sales of USD 41.5 billion and net income of USD 8.2 billion. Approximately USD 7.2 billion was invested in R&D activities throughout the Group. Headquartered in Basel, Switzerland, Novartis Group companies employ approximately 99,000 full-time-equivalent associates and operate in more than 140 countries around the world. [25,26,27,28]

Drug In Clinical Trial:-

Phases

Clinical trials involving new drugs are commonly classified into four phases. Each phase of the drug approval process is treated as a separate clinical trial. The drug-development process will normally proceed through all four phases over many years. If the drug successfully passes through Phases I, II, and III, it will usually be approved by the national regulatory authority for use in the general population. Phase IV are 'post-approval' studies. Before pharmaceutical companies start clinical trials on a drug, they conduct extensive pre-clinical studies. It involves in vitro (test tube or cell culture) and in vivo (animal) experiments using wide-ranging doses of the study drug to obtain preliminary efficacy, toxicity and pharmacokinetic information.

Such tests assist pharmaceutical companies to decide whether a drug candidate has scientific merit for further development as an investigational new drug. [24,26,27]

Phase 0

Phase 0 is a recent designation for exploratory, first-in-human trials conducted in accordance with the United States Food and Drug Administration's (FDA) 2006 Guidance on Exploratory Investigational New Drug (IND) Studies.[19] Phase 0 trials are also known as humanmicrodosing studies and are designed to speed up the development of promising drugs or imaging agents by establishing very early on whether the drug or agent behaves in human subjects as was expected from preclinical studies. Distinctive features of Phase 0 trials include the administration of single subtherapeutic doses of the study drug to a small number of subjects (10 to 15) to gather preliminary data on the agent's pharmacodynamics (what the drug does to the body) and pharmacokinetics (what the body does to the drugs).

A Phase 0 study gives no data on safety or efficacy, being by definition a dose too low to cause any therapeutic effect. Drug development companies carry out Phase 0 studies to rank drug candidates in order to decide which has the best pharmacokinetic parameters in humans to take forward into further development. They enable go/no-go decisions to be based on relevant human models instead of relying on sometimes inconsistent animal data. Questions have been raised by experts about whether Phase 0 trials are useful, ethically acceptable, feasible, speed up the drug development process or save money, and whether there is room for improvement. [28]

Phase I

Trials are the first stage of testing in human subjects. Normally, a small (20-100) group of healthy volunteers will be selected. This phase includes trials designed to assess the safety (pharmacovigilance), tolerability, pharmacokinetics, and pharmacodynamics of a drug. These trials are often conducted in an inpatient clinic, where the subject can be observed by full-time staff. The subject who receives the drug is usually observed until several half-lives of the drug have passed. Phase I trials also normally include dose-ranging, also called dose escalation, studies so that the appropriate dose for therapeutic use can be found. The tested range of doses will usually be a fraction of the dose that causes harm in animal testing. Phase I trials most often include healthy volunteers. However, there are some circumstances when real patients are used, such as patients who have terminal cancer or HIV and lack other treatment options. "The reason for conducting the trial is to discover the point at which a compound is too poisonous to administer." Volunteers are paid an inconvenience fee for their time spent in the volunteer centre. Pay ranges from a small amount of money for a short period of residence, to a larger amount of up to approx $6000 depending on length of participation.

There are different kinds of Phase I trials: [29]

(a) SAD

Single Ascending Dose studies are those in which small groups of subjects are given a single dose of the drug while they are observed and tested for a period of time. If they do not exhibit any adverse side effects, and the pharmacokinetic data is roughly in line with predicted safe values, the dose is escalated, and a new group of subjects is then given a higher dose. This is continued until pre-calculated pharmacokinetic safety levels are reached, or intolerable side effects start showing up (at which point the drug is said to have reached the Maximum tolerated dose (MTD). [27,29,30]

(b) MAD

Multiple Ascending Dose studies are conducted to better understand the pharmacokinetics & pharmacodynamics of multiple doses of the drug. In these studies, a group of patients receives multiple low doses of the drug, while samples (of blood, and other fluids) are collected at various time points and analyzed to understand how the drug is processed within the body. The dose is subsequently escalated for further groups, up to a predetermined level. [30,31]

Food effect

A short trial designed to investigate any differences in absorption of the drug by the body, caused by eating before the drug is given. These studies are usually run as a crossover study, with volunteers being given two identical doses of the drug on different occasions; one while fasted, and one after being fed. [31,32]

Phase II

Once the initial safety of the study drug has been confirmed in Phase I trials, Phase II trials are performed on larger groups (20-300) and are designed to assess how well the drug works, as well as to continue Phase I safety assessments in a larger group of volunteers and patients. When the development process for a new drug fails, this usually occurs during Phase II trials when the drug is discovered not to work as planned, or to have toxic effects.

Phase II studies are sometimes divided into Phase IIA and Phase IIB.

- Phase IIA is specifically designed to assess dosing requirements (how much drug should be given).
- Phase IIB is specifically designed to study efficacy (how well the drug works at the prescribed dose(s))

Some trials combine Phase I and Phase II, and test both efficacy and toxicity.

Trial design

Some Phase II trials are designed as case series, demonstrating a drug's safety and activity in a selected group of patients. Other Phase II trials are designed as randomized clinical trials, where some patients receive the drug/device and others receive placebo/standard treatment. Randomized Phase II trials have far fewer patients than randomized Phase III trials. [33,34,35,36]

Phase III

Phase III studies are randomized controlled multicenter trials on large patient groups (300–3,000 or more depending upon the disease/medical condition studied) and are aimed at being the definitive assessment of how effective the drug is, in comparison with current 'gold standard' treatment. Because of their size and comparatively long duration, Phase III trials are the most expensive, time-consuming and difficult trials to design and run, especially in therapies for chronic medical conditions.

It is common practice that certain Phase III trials will continue while the regulatory submission is pending at the appropriate regulatory agency. This allows patients to continue to receive possibly lifesaving drugs until the drug can be obtained by purchase. Other reasons for performing trials at this stage include attempts by the sponsor at "label expansion" (to show the drug works for

additional types of patients/diseases beyond the original use for which the drug was approved for marketing), to obtain additional safety data, or to support marketing claims for the drug. Studies in this phase are by some companies categorised as "Phase IIIB studies."[23,24,38]

While not required in all cases, it is typically expected that there be at least two successful Phase III trials, demonstrating a drug's safety and efficacy, in order to obtain approval from the appropriate regulatory agencies such as FDA (USA), or the EMA (European Union), for example.Once a drug has proved satisfactory after Phase III trials, the trial results are usually combined into a large document containing a comprehensive description of the methods and results of human and animal studies, manufacturing procedures, formulation details, and shelf life. This collection of information makes up the "regulatory submission" that is provided for review to the appropriate regulatory authorities in different countries. They will review the submission, and, it is hoped, give the sponsor approval to market the drug. Most drugs undergoing Phase III clinical trials can be marketed under FDA norms with proper recommendations and guidelines, but in case of any adverse effects being reported anywhere, the drugs need to be recalled immediately from the market. While most pharmaceutical companies refrain from this practice, it is not abnormal to see many drugs undergoing Phase III clinical trials in the market.[25,35,37]

Phase IV

Phase IV trial is also known as Post-Marketing Surveillance Trial. Phase IV trials involve the safety surveillance (pharmacovigilance) and ongoing technical support of a drug after it receives permission to be sold. Phase IV studies may be required by regulatory authorities or may be undertaken by the sponsoring company for competitive (finding a new market for the drug) or other reasons (for example, the drug may not have been tested for interactions with other drugs, or on certain population groups such as pregnant women, who are unlikely to subject themselves to trials). The safety surveillance is designed to detect any rare or long-term adverse effects over a much larger patient population and longer time period than was possible during the Phase I-III clinical trials. Harmful effects discovered by Phase IV trials may result in a drug being no longer sold, or restricted to certain uses: recent examples involve cerivastatin (brand names Baycol and Lipobay), troglitazone(Rezulin) and rofecoxib (Vioxx). [39]

References

1. In severe congestive heart failure. Results of the Cooperative North Scandinavian Enalapril Survival Study (CONSENSUS). N Engl J Med 1987;316:1429–35.
2. The SOLVD Investigators. Effect of enalapril on survival in patients with reduced left ventricular ejection fractions and congestive heart failure. The SOLVD investigators. N Engl J Med 1991;325:293–302.
3. Pitt B, Poole-Wilson PA, Segal R. Effect of losartan compared with captopril on mortality in patients with symptomatic heart failure randomised trial—the Losartan Heart Failure Survival Study ELITE II. Lancet 2000;355:1582–7.
4. Cohn JN, Tognoni G. Valsartan Heart Failure Trial Investigators. A randomized trial of the angiotensin-receptor blocker valsartan in chronic heart failure. N Engl J Med 2001;345:1667–75.
5. Pfeffer MA, Swedberg K, Granger CB, et al. Effects of candesartan on mortality and morbidity in patients with chronic heart failure: theCHARM-Overall programme. Lancet 2003;362:759–66.

6. Boger RS, Glassman HN, Cavanaugh JH, et al. Prolonged duration of blood pressure response to enalkiren, the novel dipeptide rennin inhibitor, in essential hypertension. Hypertension 1990;15:835–40.
7. MacFadyen RJ, Jones CR, Doig JK, Birnbock H, Reid JL. Responses to an orally active renin inhibitor, remikiren (Ro 42-5892), after controlled salt depletion in humans. J Cardiovasc Pharmacol 1995;25:347–53.
8. Neuberg GW, Kukin ML, Penn J, et al. Hemodynamic effects of rennin inhibition by enalkiren in chronic congestive heart failure. Am J Cardiol 1991;67:63–6.
9. Stanton A, Jensen C, Nussberger J, O'Brien E. Blood pressure lowering in essential hypertension with an oral renin inhibitor, aliskiren. Hypertension 2003;42:1137–43.
10. Gradman AH, Schmieder RE, Lins RL, et al. Aliskiren, a novel orally effective renin inhibitor, provides dose-dependent antihypertensive efficacy and placebo-like tolerability in hypertensive patients. Circulation 2005;111:1012–8.
11. The CONSENSUS Trial Study Group. Effects of enalapril on mortality Gradman A, Schmieder R, Lins R, Nussberger J, Chiang Y, Bedigian M (2005). "Aliskiren, a novel orally effective renin inhibitor, provides dose-dependent antihypertensive efficacy and placebo-like tolerability in hypertensive patients". *Circulation* 111 (8): 1012–8. doi:10.1161/01.CIR.0000156466.02908.ED. PMID 15723979.
12. Straessen JA, Li Y, and Richart T (2006). "Oral Renin Inhibitors". *Lancet* 368 (9545): 1449–56. doi:10.1016/S0140-6736(06)69442-7. PMID 17055947. http://linkinghub.elsevier.com/ retrieve/ pii/S0140673606694427.
13. "First Hypertension Drug to Inhibit Kidney Enzyme Approved". CBC. 2007-03-06. http://www.cbc.ca/cp/HealthScout/070306/6030611AU.html. Retrieved 2007-03-14.
14. "Chemistry & Biology : Structure-based drug design: the discovery of novel nonpeptide orally active inhibitors of human renin". Science Direct. http://www.sciencedirect.com/science?_ob=ArticleURL&_udi=B6VRP-40S0BHP&_user=10&_rdoc=1&_fmt=&_orig=search&_sort=d&view=c&_version=1&_urlVersion=0&_userid=10&md5=cd88f72d4f6d5568c07664397a92b584. Retrieved 2010-01-20.
15. Dzau V. The cardiovascular continuum and renin-angiotensinaldosterone system blockade. *J Hypertens Suppl* 2005; 23: S9–17.
16. Kim S, Iwao H. Molecular and cellular mechanisms of angiotensin II-mediated cardiovascular and renal diseases. *Pharmacol Rev* 2000; 52: 11–34.
17. Buhler FR, Laragh JH, Baer L, Vaughan ED Jr, Brunner HR. Propranolol inhibition of renin secretion: a specifi c approach to diagnosis and treatment of renin-dependent hypertensive diseases. *N Engl J Med* 1972; 287: 1209–14.
18. Ondetti MA, Rubin B, Cushman DW. Design of specifi c inhibitors of angiotensin-converting enzyme: new class of orally active antihypertensive agents. *Science* 1977; 196: 441–44.
19. Tenenbaum A, Grossman E, Shemesh J, F isman EZ, N osrati I, Motro M. Intermediate but not low doses of aspirin can suppress angiotensin-converting enzyme inhibitor-induced cough. *Am J Hypertens* 2000; 13: 776–82.
20. Nussberger J, Cugno M, Amstutz C, Cicardi M, Pellacani A, Agostoni A. Plasma bradykinin in angio-oedema. *Lancet* 1998; 351: 1693–97.
21. Brunner HR, Gavras H, Laragh JH, Keenan R. Hypertension in man. Exposure of the renin and sodium components using angiotensin II blockade. *Circ Res* 1974; 24 (suppl I): I35–I43
22. Fisher NDL, Hollenberg NK. Renin inhibition: what are the therapeutic opportunities? *J Am Soc Nephrol* 2005; 16: 592–99.
23. Wood JM, Cumin F, Maibaum J. Pharmacology of renin inhibitors and their application to the treatment of hypertension. *Pharmacol Ther* 1994; 61: 325–44.

24. Hollenberg NK, Fisher ND, Price DA. Pathways for angiotensin II generation in intact human tissue: evidence from comparative pharmacological interruption of the renin system. *Hypertension* 1998; 32: 387–92.
25. Azizi M, Menard J, Bissery A, et al. Pharmacologic demonstration of the synergistic effects of a combination of the renin inhibitor aliskiren and the AT1-receptor antagonist valsartan on the angiotensin II-renin feedback interruption. *J Am Soc Nephrol.* 2004;15:3126–33.
26. Azizi M, Webb R, Nussberger J, et al. Renin inhibition with aliskiren: where are we now, and where are we going? *J Hypertens.* 2006;24:243–56.
27. Brunner HR, Gavras H, Laragh JH, et al. Hypertension in man. Exposure of the renin and sodium components using angiotensin II blockade. *Circ Res.* 1974;24(Suppl I):I35–I43.
28. Casas JP, Chua W, Loukogeorgakis S, et al. Effect of inhibitors of the renin-angiotensin system and other antihypertensive drugs on renal outcomes: systematic review and meta-analysis. *Lancet.* 2005;366:2026–33.
29. Dahlöf B, Devereux RB, Kjeldsen SE, et al. Cardiovascular morbidity and mortality in the Losartan Intervention for Endpoint reduction in hypertension study (LIFE): a randomised trial against atenolol. *Lancet.* 2002;359:995–1003.
30. Azizi M and Menard J (2004) Combined blockade of the renin-angiotensin system with Bangiotensin-converting enzyme inhibitors and angiotensin II type 1 receptor antagonists *Circulation* 109:2492–2499.
31. Azizi M, Webb R, Nussberger J, and Hollenberg NK (2006) Renin inhibition with aliskiren: where are we now, and where are we going? *J Hypertens* 24:243–256.
32. Botta L, Gerber H-U, and Schmid K (1985) Measurement of radioactivity in biological experiments, in *Drug Fate and Metabolism, Methods and Techniques* (Garrett ER and Hirtz JL eds) Vol. 5, pp 99–134, Dekker, New York.
33. Cascorbi I (2006) Role of pharmacogenetics of ATP-binding cassette transporters in the pharmacokinetics of drugs. *Pharmacol Ther* 112:457–473.
34. Centers for Disease Control and Prevention (2005) Racial/ethnic disparities in prevalence
35. Mancia G, et al. Reappraisal of European Guidelines on Hypertension Management: A European Society of Hypertension Taskforce Document J of Hypertens November 2009:27:000?.
36. Rasilez Summary of Product Characteristics (SmPC) for European Union.
37. Azizi M, Webb R, Nussberger J, Hollenberg NK. Renin Inhibition with Aliskiren: Where Are we Now, and Where Are we Going? J Hypertens 2006;24:243
38. Parving H-H, et al. Aliskiren Combined with Losartan in Type 2 Diabetes and Nephropathy. N Eng J Med June 5, 2008;358:2433-46.
39. McMurray JJ, et al. Effects of the Oral Direct Inhibitor Aliskiren in Patients with Symptomatic Heart Failure. Circulation - Heart Failure. 2008;1:17-24.
40. Solomon S, et al. Effect of the Direct Renin Inhibitor Aliskiren, Either Alone or in Combination With Losartan, Compared to Losartan, on Left Ventricular Mass in Patients With Hypertension and Left Ventricular Hypertrophy: The Aliskiren Left Ventricular Assessment of Hypertrophy (ALLAY) Trial. Circulation.2009:119: 530-537.
41. Duprez DA et al. The AGELESS Study: The Effect of Aliskiren vs Ramipril Alone or in Combination with Hydrochlorothiazide and Amlodipine in Patients ≥ 65 Years of Age with Systolic Hypertension. Oral Presentation at the American Heart Association 2008 Scientific Sessions.
42. IMS Midas Worldwide Sales Data. May 2009.