CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

200175

PHARMACOLOGY REVIEW(S)

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

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application number:	200,175
Supporting document/s:	None
Applicant's letter date:	September 30, 2009
CDER stamp date:	September 30, 2009
Product:	Tribenzor® Tablets
Drug substance:	Olmesartan medoxomil, amlodipine besylate and hydrochlorothiazide
Indication:	Hypertension
Applicant:	Daiichi Sankyo Pharma Development
Review Division:	Cardiovascular and Renal Products
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1 Executive Summary

Background

The rationale for combining two or more antihypertensive agents from different pharmacologic classes is based on the expectation that the combination will exert an additive or synergistic antihypertensive effect when compared to single drug treatment. Such combinations permit simultaneous targeting of multiple physiological systems involved in the regulation of blood pressure. This increases the chances of achieving a greater reduction in blood pressure in a short period and at lower doses of the individual components. In addition, combining two or more agents may improve patient compliance and enhance tolerability by reducing the incidence of certain side effects that are more prevalent when the drugs are used alone. The seventh report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure (2004) has emphasized the importance of achieving blood pressure goals through aggressive treatment with multiple medications, if needed.

The current NDA is a 505(b)(2) application describes the efficacy and safety of the fixed-dose combination of olmesartan medoxomil, amlodipine and hydrochlorothiazide [CS8635 (b)(4)] in the treatment of essential hypertension. Olmesartan medoxomil is a non-peptidic, orally effective, specific antagonist of angiotensin II, active at the AT-1 receptor. Amlodipine is a calcium ion influx inhibitor of the dihydropyridine group. It inhibits the transmembrane influx of calcium ions into cardiac and vascular smooth muscle, which results in peripheral arterial vasodilatation, reduction in peripheral vascular resistance and reduction in blood pressure. Hydrochlorothiazide is a thiazide diuretic. It affects the renal tubular mechanisms of electrolyte reabsorption, directly increasing excretion of sodium and chloride in approximately equivalent amounts. All three drugs have been extensively studied and are widely used as monotherapies for the treatment of hypertension. Since these three classes of agents have different modes of action, their combination might provide an additive or synergistic antihypertensive effect when compared to single drug treatment.

1.1 Recommendations

1.1.1 Approvability

Approvable

1.1.2 Additional Non Clinical Recommendations

None

1.1.3 Labeling

Those sections of the proposed labeling (EDR version dated September 10, 2009) that deal with nonclinical studies covered by this review are considered satisfactory with the following exceptions.

8. USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

a) To be consistent with the new labeling requirements, the nonclinical data in section 8.1 should be moved to section 13.3.

b) The information on hydrochlorothiazide given at the end of the animal data should be moved up. Additional information pointing toward section 13.3 will be now 6th paragraph in section 8.1. Both 5th and 6th paragraphs for the section 8.1 are as follows with our recommended changes underlined.

Thiazides cross the placental barrier and appear in cord blood. There is a risk of fetal or neonatal jaundice, thrombocytopenia and possibly other adverse reactions that have occurred in adults.

Healthcare professionals who prescribe drugs acting directly on the renin angiotensin aldosterone system should counsel women of childbearing potential about the risks of these agents during pregnancy. [See Nonclinical Toxicology (13.3)]

10. OVERDOSAGE

The sponsor text does not include rodent-to-human- mg/m^2 dosage multiples for HCTZ. The following statement incorporates our recommended changes (underlined).

Hydrochlorothiazide. The most common signs and symptoms of overdose observed in humans are those caused by electrolyte depletion (hypokalemia, hypochloremia, hyponatremia) and dehydration resulting from excessive diuresis. If digitalis has also been administered, hypokalemia may accentuate cardiac arrhythmias. The degree to which hydrochlorothiazide is removed by hemodialysis has not been established. The oral LD_{50} of hydrochlorothiazide is greater than 10 g/kg in both mice and rats. (b) (4)

13. 1 Carcinogenesis, Mutagenesis, Impairment of Fertility

The sponsor has not conducted carcinogenicity studies with the triple combination. The sponsor text does not include rodent-to-human- mg/m^2 dosage multiples for olmesartan medoxomil and HCTZ. The following statement incorporates our recommended changes (underlined).

Studies with Olmesartan medoxomil, Amlodipine and Hydrochlorothiazide. No carcinogenicity, mutagenicity or fertility studies have been conducted with the combination of olmesartan medoxomil, amlodipine and hydrochlorothiazide. However, these studies have been conducted for olmesartan medoxomil, amlodipine and hydrochlorothiazide alone.

Studies with Olmesartan medoxomil. Olmesartan was not carcinogenic when administered by dietary administration to rats for up to 2 years. The highest dose tested (2000 mg/kg/day) was, on a mg/m^2 basis, about 480 times the MRHD of 40 mg/day. Two carcinogenicity studies conducted in mice, a 6-month gavage study in the p53 knockout mouse and a 6-month dietary

administration study in the Hras2 transgenic mouse, at doses of up to 1000 mg/kg/day (on a mg/m^2 basis, about 120 times the MRHD of 40 mg/day), revealed no evidence of a carcinogenic effect of olmesartan.

Both olmesartan medoxomil and olmesartan tested negative …….. up to 2000 mg/kg (olmesartan not tested).

Fertility of rats was unaffected by administration of olmesartan at dose levels as high as 1000 mg/kg/day (240 times the MRHD of 40 mg/day on a mg/m^2 basis) in a study in which dosing was begun 2 (female) or 9 (male) weeks prior to mating. (Calculations based on a 60 kg patient.)

Studies with Amlodipine. Rats and mice……on a 60 kg patient).

Studies with Hydrochlorothiazide. Two-year feeding studies in mice and rats conducted under the auspices of the National Toxicology Program (NTP) uncovered no evidence of a carcinogenic potential of hydrochlorothiazide in female mice (at doses of up to approximately 600 mg/kg/day) or in male and female rats (at doses of up to approximately 100 mg/kg/day). These doses in mice and rats are about 117 and 39 times, respectively, the MRHD of 25 mg/day on a mg/m^2 basis. (Calculations based on a 60 kg patient.) The NTP, however, found equivocal evidence for hepatocarcinogenicity in male mice.

Hydrochlorothiazide was not genotoxic *in vitro* in the Ames mutagenicity assay of *Salmonella typhimurium* strains TA 98, TA 100, TA 1535, TA 1537, and TA 1538, or in the Chinese Hamster Ovary (CHO) test for chromosomal aberrations. It was also not genotoxic *in vivo* in assays using mouse germinal cell chromosomes, Chinese Hamster bone marrow chromosomes, or in *Drosophilla* sex-linked recessive lethal trait gene. Positive test results were obtained in the *in vitro* CHO Sister Chromatid Exchange (clastogenicity) assay, the Mouse Lymphoma Cell (mutagenicity) assay and the *Aspergillus nidulans* nondisjunction assay.

Hydrochlorothiazide had no adverse effects on the fertility of mice and rats of either sex in studies wherein these species were exposed, via their diet, to doses of up to 100 and 4 mg/kg, respectively, prior to mating and throughout gestation. These doses in mice and rats are about 19 and 1.5 times, respectively, the MRHD of 25 mg/day on a mg/m^2 basis. (Calculations based on a 60 kg patient.)

13.3 Developmental Toxicity

To be consistent with the new labeling requirements, the nonclinical data placed in section 8.1 is moved to section 13.3. The sponsor text for rodent-to-human- mg/m^2 dosage multiples for olmesartan medoxomil and HCTZ are incorrect. The sponsor text is reproduced below with our recommended changes (underlined).

Studies with Olmesartan medoxomil, Amlodipine and Hydrochlorothiazide. No reproductive studies have been conducted with the combination of olmesartan medoxomil, amlodipine and hydrochlorothiazide. However, these studies have been conducted for olmesartan medoxomil,

amlodipine and hydrochlorothiazide alone, and olmesartan medoxomil and hydrochlorothiazide together.

Studies with Olmesartan medoxomil. No teratogenic effects were observed……..MRHD of 40 mg/day.

Studies with Olmesartan medoxomil and Hydrochlorothiazide. No teratogenic effects were observed when 1.6:1 combinations of olmesartan medoxomil and hydrochlorothiazide were administered to pregnant mice at oral doses up to 1625 mg/kg/day (122 times the MRHD on a mg/m^2 basis) or pregnant rats up to 1625 mg/kg/day (243 times the MRHD on a mg/m^2 basis) or pregnant rabbits at oral doses up to 1 mg/kg/day (0.3 times the MRHD on a mg/m^2 basis). In rats, however, fetal body weights at 1625 mg/kg/day (a toxic, sometimes lethal dose in the dams) were significantly lower than control. The no observed effect dose for developmental toxicity in rats is 162.5 mg/kg/day, about 24 times, on a mg/m^2 basis, the MRHD of 40 mg olmesartan medoxomil/25 mg hydrochlorothiazide/day. (Calculations based on a 60 kg patient.)

Studies with Amlodipine. No evidence of teratogenicity…….potential risk to the fetus.

Studies with Hydrochlorothiazide. Thiazides cross the……in adults.

1.2 Brief Discussion of Nonclinical Findings

The sponsor has not performed pharmacology or ADME studies for the combination product. To support the chronic administration of the olmesartan medoxomil (OM), amlodipine besylate (AML) and hydrochlorothiazide (HCTZ) combination (CS8635) to adult hypertensive patients, a 3 month repeat dose toxicity study was performed in F344 rats.

In this study, OM, HCTZ and AML were administered orally, by gavage, together, at three different ratios of 10:6.25:1; 5:3.13:1; 1.5:0.94:1 (OM:HCTZ:AML) on a weight basis. Two additional groups received either OM and HCTZ (10:6.25) or AML alone. (All doses and dose ratios in this review are presented in terms of the amlodipine base.) It may be noted that the clinical ratios are different, viz. 4:2.5:1; 8:2.5:1; 8:5:1 (OM:HCTZ:AML)

In this study 2 deaths occurred, neither of which were attributed to drug treatment. The target organ toxicities in both sexes were the kidney (thickening of the arterial wall of the afferent arterioles/interlobular arteries and regeneration of tubules), stomach (submucosal fibrosis in the pylorus), intestine (diffuse mucosal thickening in the cecum and colon) and adrenal (vacuolation of the fascicular cortical cells), and in females the ovary (vacuolation of the lutein cells), uterus and vagina (atrophy). Additional findings included a statistically significant increase in BUN (>2-fold), decrease in erythroid parameters, and dose-dependent decreases in mean body weight gain and food consumption relative to concurrent control for both sexes at all dose combinations. Most of these findings (except for effects on the adrenal and female sex organs) were observed with OM alone at a much higher dose (100 or more mg/kg/day) in previous studies (see NDA 21,286 for OM; NDA 21,532 for OM/HCTZ; NDA 22,100 for OM/AML) than with the combined administration of OM and HCTZ (with

or without AML) in the present study. The incidence and severity of adverse effects were slightly greater for the combination than for individual components. This suggests that AML and HCTZ to some extent augment the toxicities of OM.

Toxicokinetics demonstrated accumulation of AML and a significant increase in systemic exposure to olmesartan, the active metabolite of OM, and HCTZ with the co-administration of AML besylate for either sex. This is because AML increases absorption of OM and HCTZ in the gut as a result of an excessive relaxant effect on the gastrointestinal smooth muscle. It is unlikely that the moderate systemic exposure to olmesartan and HCTZ was responsible for toxicities noted in the combination groups. Human PK studies indicated no pharmacokinetic drug-drug interactions between the active components of CS-8635 in clinical pharmacology studies, since OM and HCTZ had no effect on the AML toxicokinetic parameters.

Systemic exposures (AUCs) to olmesartan:HCTZ:AML in rats treated with the combination at the lowest dose were compared to systemic exposures in humans treated at the highest combination doses of OM (40 mg), HCTZ (25 mg) and amlodipine (10 mg). Exposures to olmesartan, HCTZ and AML in rats (the dose that resulted in toxicities noted above since a NOAEL was not established in the study) were, respectively, 3, 15 and 5 times the exposure in humans at the maximum recommended human dose. The combination product can be used safely in humans for the treatment of hypertension because the target organ toxicities are monitorable and attributable to the individual drugs of the combination, drugs which are currently approved for use in this patient population and have often been used concomitantly.

2 Drug Information

2.1 **Drug Product**: Tribenzor® Tablets (CS-8635)

2.2 **Drug Substances**

Generic name: **Olmesartan Medoxomil**

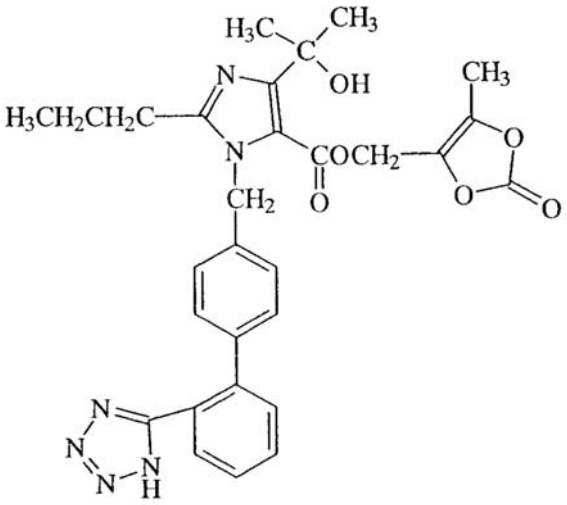
Code name: CS-866, RNH-6334

Chemical name: (5-Methyl-2-oxo-1,3-dioxol-4-yl)methyl 4-(hydroxy-1-methylethyl)-2-propyl-1-[[2'-(1H-tetrazol-5-yl)-1,1'-biphenyl-4-yl]methyl]-1H-imidazole-5-carboxylate

Chemistry: Olmesartan medoxomil is an imidazole with no chiral center. It is a white to pale yellowish white powder, practically insoluble in water and sparingly soluble in organic solvents such as methanol and acetone. It is not hygroscopic.

CAS registry number: 144689-63-4

Molecular formula/molecular weight: $C_{29}H_{30}N_6O_5$/ 558.59



Generic name: **Amlodipine Besylate**

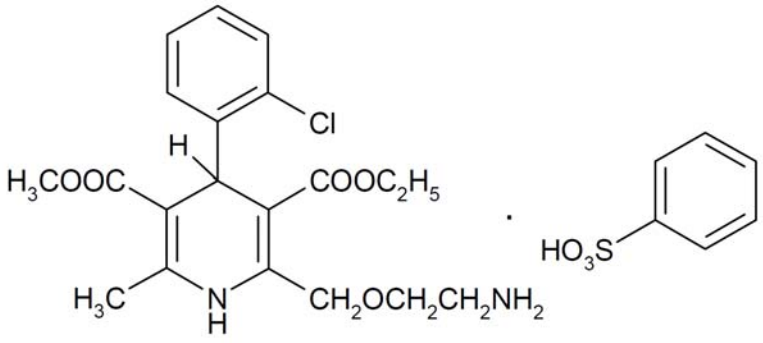
Code name: LBT873-DMA.002

Chemical name: (*RS*)-2-[(2'-aminoethoxy)methyl]-4-(2-chlorophenyl)-1,4-dihydro-6-methyl-3,5-pyridinedicarboxylic acid 3-ethyl ester, 5-methyl ester, benzene sulfonate.

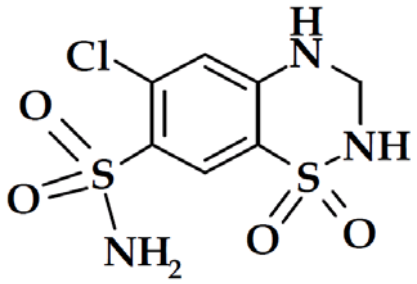
Chemistry: Amlodipine is a racemic mixture (R and S isomers). It is a white to pale yellow crystalline powder slightly soluble in water and sparingly soluble in ethanol.

CAS registry number: 1114790-99-6 (besylate salt form)
88150-42-9 (free base form)

Molecular formula/molecular weight: $C_{20}H_{25}ClN_2O_5 \cdot C_6H_5SO_3H$ / 567.06 (besylate)



Generic name: **Hydrochlorothiazide (HCTZ)**
Chemical name: 6-chloro-3,4-dihydro-2*H*-1,2,4-benzothiadiazine-7-sulfonamide 1,1-dioxide.
Chemistry: Hydrochlorothiazide is a white, or practically white, crystalline powder which is slightly soluble in water and freely soluble in sodium hydroxide solution.
CAS registry number: 58-93-5
Molecular formula/molecular weight: $C_7H_8ClN_3O_4S_2$ / 297.74



Related Applications: Clinical trials supporting the current NDA were conducted under Daiichi Sankyo's IND 77,651. Pfizer's NDA 19,787 for racemic amlodipine besylate (Norvasc®) was approved for the treatment of hypertension, chronic stable angina and vasospastic angina in 1992. Sankyo's NDA 21,286 for olmesartan medoxomil (Benicar®) was approved for the treatment of hypertension in April 2002. Other related NDAs are: 21,532 for the combination of OM and HCTZ, and 22,100 for the combination of OM and amlodipine.

2.3 Drug Class: Olmesartan: Angiotensin II receptor type 1 (AT_1 receptor) antagonist
Hydrochlorothiazide: Thiazide diuretic

Amlodipine: Dihydropyridine calcium channel blocker

2.4 Intended Clinical Population: Hypertensive subjects

2.5 Clinical Formulation and Dosing Regimen: The tablets are formulated in five strengths of olmesartan medoxomil/ amlodipine besylate/Hydrochlorothiazide, respectively: 20/5/12.5 mg, 40/5/12.5 mg, 40/5/25 mg, 40/10/12.5 mg and 40/10/25 mg. Table 1 lists proposed final commercial formulations.

3 Studies Submitted

1. Repeat dose toxicity study for 28 days (non-GLP study)
2. Repeat-dose toxicity study for 3 months (GLP study)

3.1 Studies Reviewed

Repeat-dose toxicity study for 3 months (GLP study)
The 28 day toxicity study is referenced in the above study.

Table 1. The composition of drug product

Component	Quality Std.	Function	20/5/12.5 mg	40/5/12.5 mg	40/5/25 mg	40/10/12.5 mg	40/10/25 mg
(b) (4)							
Olmesartan medoxomil	DMF (b) (4)	Drug substance	20.000	40.000	40.000	40.000	40.000
Amlodipine besylate	EP/USP DMF (b) (4) DMF (b) (4)	Drug substance	6.944[1]	6.944[1]	6.944[1]	13.888[1]	13.888[1]
Hydrochlorothiazide	EP/USP (b) (4) DMF DMF (b) (4)	Drug substance	12.500	12.500	25.000	12.500	25.000
Starch, pregelatinized	EP	(b) (4)					
Silicified microcrystalline cellulose[2]	DMF (b) (4)						
Croscarmellose sodium	EP						
Magnesium stearate	EP						
(b) (4)							
(b) (4)							
Total Tablet Weight (mg)			208	310	412	310	412

[1] Equivalent to 5 mg (6.944 mg) and 10 mg (13.888 mg) amlodipine

(b) (4)

(b) (4)

3.2 Studies Not Reviewed

Repeat-dose toxicity study for 28 days (non-GLP study)

3.3 Previous Reviews Referenced

NDA 21-286 for Benicar® (olmesartan medoxomil), NDA 21-532 for Benicar HCT® (olmesartan medoxomil and hydrochlorothiazide) and NDA 22-100 for Azor® (amlodipine and olmesartan medoxomil).

4 Pharmacology

No new pharmacology studies are included in this NDA.

5 Pharmacokinetics/ADME/Toxicokinetics

No new pharmacokinetics studies are included in this NDA.

6 General Toxicology

6.1 Single-Dose Toxicity

No studies conducted

6.2 Repeat-Dose Toxicity

Study title: **3 Month Oral Gavage Study in F344 Rats**

Study no.: B-6493, Report #AN08-C0045-R01 and AN08-C0093-R01 (TK)
Study report location: EDR
Conducting laboratory and location: (b) (4)

Date of study initiation: October 9 (males) and 10 (females), 2008
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: CS-8635 is a combination drug composed of: Olmesartan medoxomil, lot #0109, 98.9% pure; HCTZ, lot #17455101, 100.2% pure; Amlodipine besylate, lot #7, 100.5% pure.

Key Study Findings

The deaths of two males (one each receiving 100:62.5 mg (OM:HCTZ)/kg/day and 100:62.5:10 mg (OM:HCTZ:AML)/kg/day during the study were considered not related to an effect of the test substance. Principal drug-related findings in both sexes for all treated groups (except for amlodipine besylate alone group) relative to control were statistically significant decreases in mean body weight gain, food intake, erythroid parameters), and increases in BUN, creatinine, potassium and alkaline phosphatase. Statistically significant decreases in the thymus (relative) and increases in the stomach (absolute and relative) weights for both sexes were noted in the CS-8635 (triple combination) groups relative to control. In the females of all dose groups except AML alone, statistically significant and non-dose-dependent decreases in the pituitary, heart and uterus weights, and increases in the ovary weights relative to control were noted. Test substance related microscopic pathology was observed in the stomach (submucosal fibrosis), intestinal tract (diffuse mucosal thickening), adrenal (hypertrophy of the glomerular cells), kidney (thickening of the arterial wall and tubular regeneration), uterus and vagina (atrophy) in the CS-8635 and OM:HCTZ groups. Toxicokinetics analysis showed that Cmax and $AUC_{0\text{-}24h}$ values for olmesartan and HCTZ in the presence of AML were several fold higher than in the absence of AML, suggesting enhancement of effects of OM and HCTZ by co-administration with amlodipine besylate.

Methods

Doses: See Table 2.
Frequency of dosing: Once daily, for 13 weeks.
Route of administration: Orally by gavage using a stomach tube
Dose volume: 5 ml/kg

Formulation/Vehicle: The drugs were suspended in 0.5% (w/v) methylcellulose aqueous solution in water for injection. An equivalent volume of the concentrated suspension of each test substance was mixed to make the prescribed concentration of CS-8635 (the triple combination). It was prepared once every 7 days, and used within 7 days of preparation (stored at 2 to 10°C). All of the investigational drugs were stable for 1 day at 23°C and for more than 8 days at 4°C. The homogeneity and concentration of each dose suspension prepared in weeks 1, 4 and 13 were analyzed by taking 3 samples per concentration.

Table 2. Study design

Test group	Dose (mg/kg/day)[b)]	Concentration (mg/mL)[b)]	Dose volume (mL/kg)	Sex	Main group		Satellite group	
					No. of animals	Animal No.	No. of animals	Animal No.
Control[a)]	0/0/0	0/0/0	5	M	15	1001-1015	5	1201-1205
				F	15	1101-1115	5	1301-1305
OM/HCTZ	100/62.5/0	20/12.5/0	5	M	15	2001-2015	5	2201-2205
				F	15	2101-2115	5	2301-2305
OM/HCTZ/AML	100/62.5/10	20/12.5/2	5	M	15	3001-3015	5	3201-3205
				F	15	3101-3115	5	3301-3305
	100/62.5/20	20/12.5/4	5	M	15	4001-4015	5	4201-4205
				F	15	4101-4115	5	4301-4305
	30/18.75/20	6/3.75/4	5	M	15	5001-5015	5	5201-5205
				F	15	5101-5115	5	5301-5305
AML	0/0/20	0/0/4	5	M	15	6001-6015	5	6201-6205
				F	15	6101-6115	5	6301-6305

a): 0.5 w/v% MC solution
b): OM/HCTZ/AML
M: Male, F: Female

Species/Strain: Rats, F344/DuCrlCrlj SPF (b) (4)
Number/Sex/Group: 15/sex for toxicology
Age: 7 weeks old at initiation of dosing
Weight: Males: 131-158 gm, Females: 105-128 gm, at initiation of dosing
Satellite groups: 5/sex/dose group for toxicokinetics (see Table 2).
Unique study design: The experimental design was to evaluate the effect of amlodipine on the toxicity of OM and HCTZ. The highest dose of OM was set at 100 mg/kg/day, at which exposure level of RNH-6270 (an active metabolite of OM) was expected to exceed the highest human exposure level. Two additional groups were provided to compare the toxicity of CS-8635 (i.e., combination of three) with those of concomitant administration of OM and HCTZ and of AML alone (Table 2). Control animals received the vehicle.
Rationale for dose selection: Doses were selected on the basis of a 28 day oral toxicity study in rats (same strain) in which CS-8635 at doses of 100:62.5:20 (OM/HCTZ/AML) mg/kg/day caused suppression of body weight gain, increased urine volume, decreased red blood cell parameters, increased BUN level, regeneration of the renal tubules and thickening of the arterial wall in the kidney. Therefore, it was considered to be an appropriate dose to investigate synergistic effects of the triple combination. In addition, based on the TK results from this study, a separate group dosed with OM/HCTZ/AML at 30:18.75:20 mg/kg/day, at which exposure levels of RNH-6270 and HCTZ were expected to be comparable to those of OM/HCTZ at 100:62.5 mg/kg/day was provided.
Deviation from study protocol: None

Observations and Measurements

Only clinical signs and body weights were evaluated for toxicokinetic animals; however, these were excluded from toxicity evaluation.

Clinical Signs: All animals were observed thrice daily (twice during weekends) for clinical signs and mortality.

Body Weight and Food Consumption: Recorded for all animals, prior to dosing, twice in week 1, and thereafter once a week and on the day of necropsy.

Ophthalmology: Conducted on all main study animals once pretest and in week 13.

Urinalysis: Urine samples were collected for 24 hr from individual animals (10/sex/group) during study week 13 under deprivation of food but free access to water. The following parameters were assessed: urine volume, water intake, osmotic pressure, pH, color, protein, glucose, ketones, urobilinogen, bilirubin, blood, sodium, potassium and chloride.

Hematology[1] and Clinical Biochemistry[2]: Blood samples were collected prior to terminal necropsy (not fasted) from all surviving main study animals from the abdominal aorta under ether anesthesia.

Pathology: Animals were sacrificed by exsanguination after collecting blood samples. External appearance and all organs/tissues in the cranial, thoracic and abdominal cavities were examined. Representative samples of the protocol tissues (Table 3) were collected from all main study animals and processed for microscopic examination which was performed on the tissues from all animals in all dose groups.

Table 3. Tissues sampled for histopathological examination

adrenals* π	kidneys* π	sternum (including bone marrow)
cerebrum π,§	liver π	stomach @
cerebellum π, §	lungs (including bronchus) π	sublingual glands*
cecum π	mammary glands**(inguinal region, both sides)	submandibular glands*
colon	mesenteric lymph node	submandibular lymph nodes*
duodenum	ovaries* π	testes* π
epididymides* π	pancreas,	thoracic aorta
esophagus	parathyroids*	thymus π
eyeballs*	pituitary π	thyroids* π
femoral skeletal muscle**	prostate π	tongue
femurs**(including bone marrow)	rectum	trachea
gross lesions	sciatic nerves**	urinary bladder
Harderian glands*	seminal vesicles	uterus (both horns)* π
heart π	skin** (inguinal, both sides)	vagina
ileum @	spinal cord (lumbar)	
jejunum @	spleen π	

*: Paired organs were examined bilaterally; **: Paired organs were examined unilaterally; π: Organ weighed; §: weighed as a part of the brain; @: stomach and small intestine were weighed separately with their contents.

[1] erythrocytes, hematocrit, hemoglobin, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, reticulocytes ratio, white blood cell count, white blood cell differential, platelets, prothrombin time, activated partial thromboplastin time and fibrinogen.

[2] ALT, AST, AP, total bilirubin, total protein, albumin, globulins, A/G ratio, glucose, BUN, creatinine, sodium, potassium, chloride, calcium, inorganic phosphorus, triglycerides, total cholesterol, phospholipids.

Toxicokinetics: Systemic exposures to the test articles were evaluated by determining plasma concentrations of RNH-6270 (an active metabolite of OM), HCTZ and amlodipine in the satellite animals. Blood samples were collected from the jugular vein of each toxicokinetic animal including the control group on study day 1 (1st dose) and in weeks 4 (27th dose) and 13 (91st dose) at 0 (prior to dosing, except for day 1), 2, 4, 7 and 24 hr after dosing (5 rats/sex/treatment group/time point). The animals were killed after week 13 blood sampling and disposed off without further examination.

Results

Stability and Homogeneity: The formulation was stable for at least 8 days at 6°C and for at least 24 hr at room temperature. Mean concentrations of all samples analyzed were in the range of 96.5% to 106% of target concentrations.

Mortality: Two males died during the study; a male (#2001) receiving 100:62.5 mg (OM:HCTZ)/kg/day and another male (#3002) receiving 100:62.5:10 mg (OM:HCTZ:amlodipine)/kg/day died on days 92 and 35, respectively. These deaths were considered not related to an effect of the test substance. In these animals, no abnormalities were noted in clinical signs although their body weights were the lowest in their respective groups. Necropsy revealed food retention in the oral cavity. Histopathology revealed luminal dilatation of the esophagus and treatment-related changes in the kidney such as regeneration of the renal tubules and thickening of the arterial wall/interlobular arteries. Since these findings were also noted in scheduled sacrificed animals, the sponsor asserts that the cause of sudden death was probably due to accidental obstruction of airway in a dosing error.

Clinical Signs: No test substance-related clinical signs were noted in any animal throughout the drug administration period.

Body Weights: A statistically significant and non-dose-dependent reduction in mean body weight gain was noted for males and females (Fig. 1) for all treated groups (except for females receiving AML alone) relative to concurrent controls from study day 7 to the end of the dosing period (Table 4). The extent of the change in the terminal body weight for the triple combination (CS-8635) groups tended to be higher than that for OM:HCTZ and amlodipine groups in males and that for the OM:HCTZ group in females.

Table 4. Mean body weight change relative to control at the end of dosing period

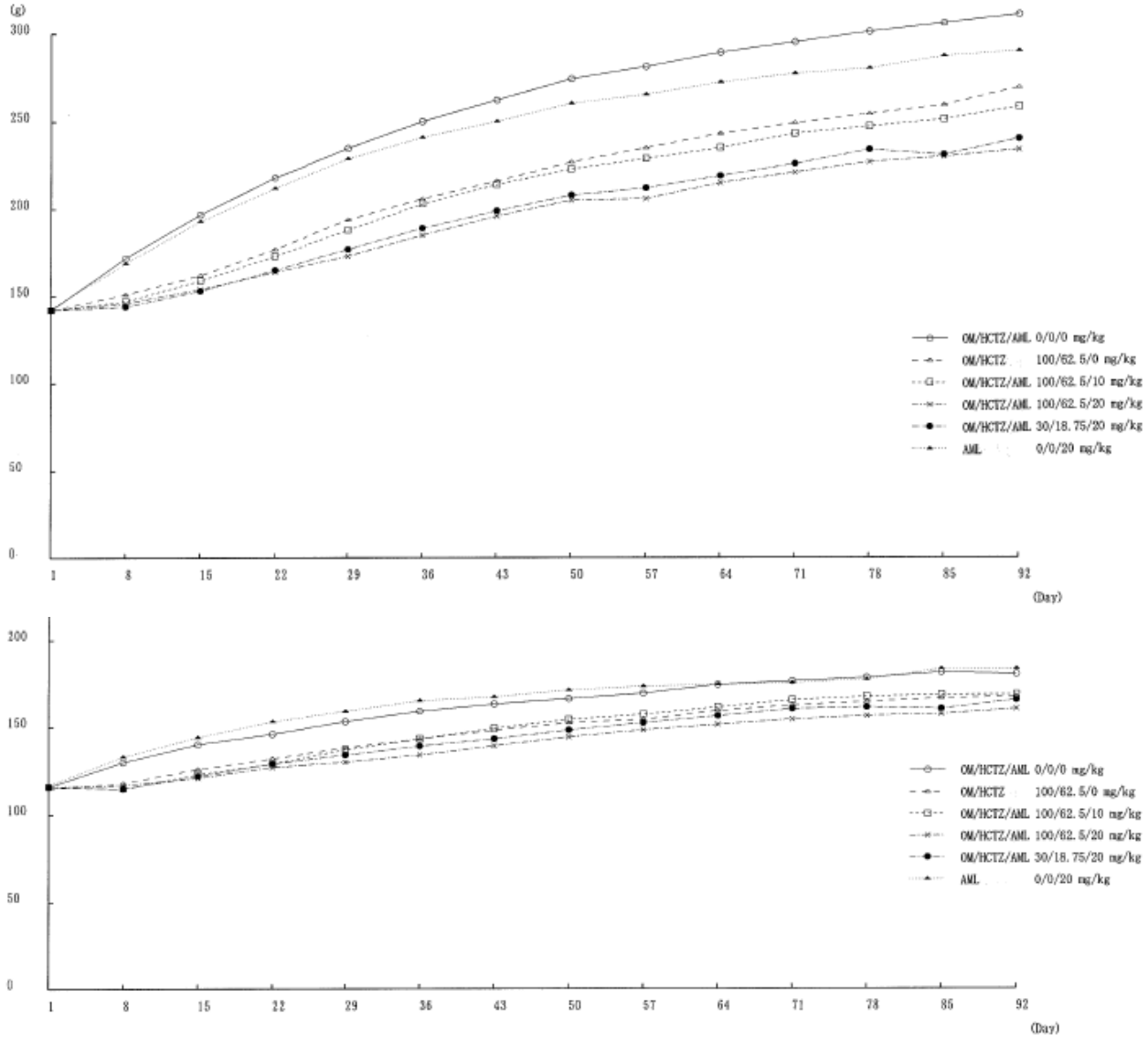
Sex	Test group	OM/HCTZ/AML Dose (mg/kg)	No. of animals	Mean body weight on Day 92
Male	OM/HCTZ	100/62.5/0	14	-14%**
	OM/HCTZ/AML	100/62.5/10	14	-17%**
		100/62.5/20	15	-25%**
		30/18.75/20	15	-23%**
	AML	0/0/20	15	-7%**
Female	OM/HCTZ	100/62.5/0	15	-7%**
	OM/HCTZ/AML	100/62.5/10	15	-7%**
		100/62.5/20	15	-11%**
		30/18.75/20	15	-8%**
	AML	0/0/20	15	N

Values in the table indicate percentage of change against the control mean (-: decrease).

N: No remarkable changes

**: $p \leq 0.01$ (significantly different from the control group)

Figure 1. Group mean body weights, males (top panel) and females (bottom panel) (treated up to 91 days). OM: Olmesartan medoxomil; HCTZ: Hydrochlorothiazide; AML: Amlodipine besylate.



Food Consumption: A statistically significant decrease in mean food consumption relative to control was noted in the CS-8635 groups at 100:62.5:20 mg (OM:HCTZ:amlodipine)/kg/day for males for the entire duration of the study and for females until day 43 of administration. At 100:62.5:10 and 100:18.75:20 mg/kg/day, statistically significant lower food consumption than control was noted for males until day 57 and for females until day 29 of administration. Thereafter, the values were comparable to that of the control group. Reduced food consumption ($p < 0.05$) was also noted for both sexes receiving OM and HCTZ, though the extent of the change was smaller than that for the CS-8635 groups. Changes in the amlodipine group were negligible since it was transient and slight.

Ophthalmoscopy: No remarkable ocular changes

Hematology: Non-dose-dependent decreases ($p < 0.05$) in RBC, hemoglobin, reticulocyte, hematocrit, prothrombin time and activated partial thromboplastin time relative to control were noted for both sexes at all combination doses and in animals receiving OM:HCTZ. Significant ($p < 0.05$) increases in MCV, MCH MCHC and platelets were noted in animals receiving the combination or receiving OM:HCTZ (Tables 5 and 6). These changes were attributed to OM and HCTZ. Other sporadic changes were considered to be negligible.

Table 5. Noteworthy findings for hematology parameters for males

Test group	OM/HCTZ	OM/HCTZ/AML			AML
OM/HCTZ/AML Dose (mg/kg/day)	100/62.5/0	100/62.5/10	100/62.5/20	30/18.75/20	0/0/20
No. of animals	14	14	15	15	15
RBC	-14%**	-15%**	-16%**	-8%**	+2%*
HGB	-10%**	-11%**	-10%**	-5%**	N
HCT	-12%**	-14%**	-13%**	-7%**	N
MCV	+2%**	+2%**	+4%**	+1%*	-2%**
MCH	+3%**	+3%**	+6%**	+3%**	-2%**
MCHC	+2%**	+2%**	+3%**	+2%**	N
Reticulocyte	-8%*	-8%	-25%**	-33%**	+21%**
PLT	+9%**	+15%**	+12%**	+8%*	+4%*
PT	-9%**	-13%**	-11%**	-8%**	N
APTT	-22%**	-30%**	-27%**	-21%**	N
WBC	+12%*	N	N	N	N
Differential leukocyte					
LYMP, ratio	N	N	N	N	-9%*
NEUT, ratio	N	N	N	N	+20%*
MONO, ratio	-18%*	-24%**	-35%**	N	N
LUC, ratio	-25%**	-25%**	-42%**	-25%**	N
LYMP, count	+15%*	N	N	N	-15%*

Values in the table indicate percentage of change against the control mean (+: increase, -: decrease).

N: No remarkable changes

* (**): $p \leq 0.05$ (0.01) (significantly different from the control group)

Table 6. Noteworthy findings for hematology parameters for females

Test group	OM/HCTZ	OM/HCTZ/AML			AML
OM/HCTZ/AML Dose (mg/kg/day)	100/62.5/0	100/62.5/10	100/62.5/20	30/18.75/20	0/0/20
No. of animals	15	15	15	15	15
RBC	-15%**	-13%**	-18%**	-6%**	+5%**
HGB	-14%**	-12%**	-16%**	-5%**	N
HCT	-15%**	-13%**	-16%**	-6%**	N
MCV	N	N	+3%**	N	-3%**
MCH	+2%**	N	+3%**	N	-4%**
MCHC	+1%*	+1%*	N	N	N
Reticulocyte	N	-24%**	-24%**	-24%**	+35%**
PLT	+12%**	N	+16%**	N	N
PT	-12%**	-12%**	-15%**	-8%**	+3%*
APTT	-21%**	-25%**	-29%**	-20%**	N
FIB	N	+8%*	N	N	-12%**
WBC	+26%**	+29%**	N	N	N
Differential leukocyte					
MONO, ratio	-29%**	-24%**	-29%**	-18%*	N
LYMP, count	+25%**	+31%**	+24%*	N	N
NEUT, count	+36%**	N	N	N	N

Values in the table indicate percentage of change against the control mean (+: increase, -: decrease).

N: No remarkable changes

* (**): $p \leq 0.05$ (0.01) (significantly different from the control group)

Clinical Chemistry: Moderate, dose-dependent increase ($p < 0.05$) in blood urea nitrogen (up to 2.7-fold), creatinine and potassium was noted for both sexes in all dose groups except for amlodipine alone. However, changes in BUN, creatinine and potassium were intensified by co-administration with AML in males. A significant increase in alkaline phosphatase relative to control was noted for both sexes in all dose groups except for females receiving amlodipine alone. Other changes were judged to be negligible since they were not dose-related, observed only at one dose level or trivial fluctuations (Tables 7 and 8).

Table 7. Noteworthy findings for clinical chemistry parameters for males

Test group	OM/HCTZ	OM/HCTZ/AML			AML
OM/HCTZ/AML Dose (mg/kg/day)	100/62.5/0	100/62.5/10	100/62.5/20	30/18.75/20	0/0/20
No. of animals	14	14	15	15	15
AST	N	N	N	N	-26%**
ALT	-18%*	-29%**	N	N	-30%**
ALP	+20%**	+23%**	+27%**	+26%**	-17%**
T-CHO	N	N	N	N	-7%**
TG	-33%*	-41%**	-57%**	-57%**	-43%**
PL	N	N	-10%**	-10%**	-13%**
GLU	N	N	N	-8%**	-6%**
BUN	+125%**	+163%**	+244%**	+100%**	N
CRNN	+12%*	+16%**	+24%**	N	N
Na	-2%**	-2%**	-1%*	-1%*	N
K	+15%**	+20%**	+33%**	+11%**	N
Cl	-1%*	N	N	N	-2%**
Ca	-4%**	-6%**	-8%**	-6%**	-4%**
P	+19%*	N	N	N	N
TP	-5%**	N	-5%**	N	-3%**
ALB	-4%*	N	N	N	N
A/G	N	N	N	N	+14%*
Globulin	-5%**	N	-8%**	N	-5%**

Table 8. Noteworthy findings for clinical chemistry parameters for females

Test group	OM/HCTZ	OM/HCTZ/AML			AML
OM/HCTZ/AML Dose (mg/kg/day)	100/62.5/0	100/62.5/10	100/62.5/20	30/18.75/20	0/0/20
No. of animals	15	15	15	15	15
ALT	N	N	N	+17%*	N
ALP	+25%**	+28%**	+50%**	+52%**	N
T-CHO	N	N	N	N	-10%*
TG	N	N	N	N	-39%**
PL	N	N	N	N	-16%**
GLU	N	N	-5%**	N	N
BUN	+213%**	+206%**	+269%**	+131%**	N
CRNN	+27%**	+23%**	+38%**	+15%**	N
Na	-1%**	-1%**	-1%**	-1%**	-1%**
K	+22%**	+20%**	+33%**	+16%**	N
Cl	-3%**	-3%**	N	-4%**	-3%**
Ca	-2%**	-4%**	-5%**	-4%**	-4%**
P	+38%**	N	N	N	N
TP	-5%**	-3%*	-5%**	N	-6%**
ALB	-4%**	-4%**	-4%**	N	-4%**
A/G	N	-13%*	N	N	N
Globulin	-6%**	N	-6%**	N	-8%**

Values in the table indicate percentage of change against the control mean (+: increase, -: decrease).

N: No remarkable changes

* (**): $p \leq 0.05$ (0.01) (significantly different from the control group)

Urinalysis: A statistically significant, non-dose-dependent mean increase in urine volume and water intake, and dose-dependent mean decrease in osmotic pressure relative to control was noted for both sexes at all combination doses (Tables 9 and Table 10). A decrease in pH was noted for both sexes in all groups. Similar changes were noted for both sexes dosed with OM:HCTZ and males dosed with AML alone. Changes in electrolyte excretion were not consistent across groups and were considered not biologically significant.

Table 9. Statistically significant changes in urine chemistry values for male rats

Test group		Control	OM/HCTZ	OM/HCTZ/AML			AML
OM/HCTZ/AML Dose (mg/kg/day)		0/0/0	100/62.5/0	100/62.5/10	100/62.5/20	30/18.75/20	0/0/20
No. of animals		10	10	10	10	10	10
pH[a]	9.0	3	0	0	0	0	1
	8.5	7	1	0	0	0	1
	8.0	0	8	2	0	1	5
	7.5	0	1	4	3	6	1
	7.0	0	0	4	6	3	1
	6.5	0	0	0	1	0	0
	6.0	0	0	0	0	0	1
Urine volume[b]		/	+223%**	+247%**	+174%**	+142%**	+133%**
Water intake[b]		/	+57%**	+57%**	+33%**	+33%**	+57%**
Osmotic pressure[b]		/	-58%**	-61%**	-61%**	-58%**	-52%**
Na[b]		/	N	N	N	N	+38%**
K[b]		/	+33%*	+27%*	N	N	N
Cl[b]		/	+30%*	N	N	N	+40%**

Table 10. Statistically significant changes in urine chemistry values for female rats

Test group		Control	OM/HCTZ	OM/HCTZ/AML			AML
OM/HCTZ/AML Dose (mg/kg/day)		0/0/0	100/62.5/0	100/62.5/10	100/62.5/20	30/18.75/20	0/0/20
No. of animals		10	10	10	10	10	10
pH[a]	9.0	1	0	0	0	0	0
	8.5	3	3	0	0	0	4
	8.0	3	6	1	0	2	2
	7.5	2	1	2	3	4	1
	7.0	1	0	6	5	3	0
	6.5	0	0	1	1	1	2
	6.0	0	0	0	1	0	1
Urine volume[b]		/	+77%**	+131%**	+59%*	+87%**	N
Water intake[b]		/	+30%**	+55%**	+30%*	+40%**	N
Osmotic pressure[b]		/	-49%**	-59%**	-61%**	-52%**	-39%**
Na[b]		/	N	N	-43%**	N	N
K[b]		/	N	N	-36%**	N	N
Cl[b]		/	N	N	-44%**	-22%*	N

a): Values in the table indicate the number of animals showed corresponding pH.

b): Values in the table indicate percentage of change against the control mean (+: increase, -: decrease).

N: No remarkable changes

/: Not applicable

* (**): $p \leq 0.05$ (0.01) (significantly different from the control group)

Gross Pathology: The major finding was that of small uteri in the CS-8635 group noted in 5/15, 2/15 and 10/15 in females dosed at 30:18.75:20, 100:62.5:10 and 100:62.5:20 (OM:HCTZ:AML) mg/kg/day, respectively. The sponsor asserts that it was associated with the suppressed body weight gain and decreased absolute and relative weights in these groups.

Organ Weights: Statistically significant and dose-dependent increases in stomach weights were noted for both sexes with all dose combinations relative to control. Statistically significant and non-dose-dependent increases in mean relative (to body) intestine weights were noted for both sexes in the CS-8635 groups relative to control (Table 11 and 12).

Table 11. Noteworthy findings for organ weights in males

Test group		OM/HCTZ	OM/HCTZ/AML			AML
OM/HCTZ/AML Dose (mg/kg/day)		100/62.5/0	100/62.5/10	100/62.5/20	30/18.75/20	0/0/20
No. of animals		14	14	15	15	15
Body weight at necropsy		-14%**	-17%**	-25%**	-23%**	-7%**
Brain	absolute	-2%*	-4%**	-5%**	-4%**	N
	relative	+15%**	+16%**	+26%**	+26%**	+6%**
Pituitary	absolute	-14%**	-21%**	-29%**	-23%**	-9%*
	relative	N	N	N	N	N
Thyroid	absolute	-17%**	-17%**	-25%**	-26%**	-17%**
	relative	N	N	N	N	-10%*
Thymus	absolute	-13%**	-29%**	-34%**	-27%**	N
	relative	N	-12%**	-12%**	N	N
Heart	absolute	-18%**	-16%**	-19%**	-18%**	+11%**
	relative	-4%**	N	+8%**	+8%**	+24%**
Lung	absolute	-4%*	-6%**	-13%**	-12%**	N
	relative	+10%**	+13%**	+17%**	+13%**	+7%**
Liver	absolute	-10%**	N	-20%**	-11%**	N
	relative	+4%**	+14%**	+7%*	+16%**	+5%**
Spleen	absolute	N	-12%**	-20%**	-17%**	N
	relative	+11%**	+5%*	N	+5%*	N
Stomach (with contents)						
	absolute	N	+42%**	+52%**	+27%	N
	relative	N	+72%**	+103%**	+66%**	N
Cecum (with contents)						
	absolute	N	N	N	N	N
	relative	+11%*	+20%**	+23%**	N	N
Kidney	absolute	N	N	-6%**	N	N
	relative	+22%**	+25%**	+25%**	+33%**	+7%**
Adrenal	absolute	N	N	N	N	N
	relative	+15%**	+15%**	+23%**	+23%**	N
Testis	absolute	-7%**	-5%**	-8%**	-6%**	N
	relative	+8%*	+15%**	+22%**	+23%**	+8%**
Prostate	absolute	N	N	-23%**	-14%*	N
	relative	+17%*	+17%*	N	N	N
Epididymis	absolute	-9%**	-8%**	-15%**	-11%**	N
	relative	N	+11%**	+13%**	+16%**	+7%**
Small intestine (with contents)						
	absolute	N	N	N	+9%*	N
	relative	+11%**	+23%**	+35%**	+43%**	N

Values in the table indicate percentage of change against the control mean (+: increase, -: decrease).
N: No remarkable changes
* (**): $p \leq 0.05$ (0.01) (significantly different from the control group)

Absolute thymus weight was significantly ($p < 0.05$) decreased for both sexes in all combination groups. Relative kidney weights were statistically significantly increased for both sexes in all combination groups. In the females of all dose groups except AML alone, statistically significant and non-dose-dependent decreases in absolute and relative pituitary, heart and uterus weights, and increases in ovary weights relative to control were noted. In the AML alone group, statistically significant decreases in absolute and relative pituitary and increases in heart and ovary weights relative to control were noted. Statistically significant differences relative to control were observed in several organs of the combination groups, however, the changes in absolute and relative weights were not in the same direction.

Table 12. Noteworthy findings for organ weights in females

Test group		OM/HCTZ	OM/HCTZ/AML			AML
OM/HCTZ/AML Dose (mg/kg/day)		100/62.5/0	100/62.5/10	100/62.5/20	30/18.75/20	0/0/20
No. of animals		15	15	15	15	15
Body weight at necropsy		-7%**	-7%**	-11%**	-8%**	N
Brain	absolute	N	-3%**	-5%**	-4%**	N
	relative	+6%**	+4%*	+7%**	+5%*	N
Pituitary	absolute	-27%**	-28%**	-37%**	-36%**	-17%**
	relative	-22%**	-23%**	-28%**	-30%**	-18%**
Thyroid	absolute	N	N	-21%**	N	N
	relative	N	N	N	N	N
Thymus	absolute	-11%**	-20%**	-23%**	-19%**	N
	relative	N	-14%**	-13%**	-11%**	N
Heart	absolute	-17%**	-15%**	-17%**	-13%**	+15%**
	relative	-10%**	-7%**	-7%**	-7%**	+13%**
Lung	absolute	N	N	-8%**	-7%**	N
	relative	N	+5%**	N	N	N
Liver	absolute	N	-8%*	N	N	N
	relative	N	+10%**	+11%**	+10%**	N
Spleen	absolute	N	N	-12%*	N	N
	relative	+14%**	+14%**	N	N	+9%*
Stomach (with contents)						
	absolute	N	+45%**	+62%**	+53%**	N
	relative	+29%*	+57%**	+83%**	+69%**	N
Kidney	absolute	+8%**	+11%**	N	+13%**	N
	relative	+18%**	+22%**	+18%**	+25%**	N
Adrenal	absolute	N	N	-15%**	-17%**	N
	relative	N	+4%*	N	-9%*	N
Ovary	absolute	+23%**	+43%**	+21%**	+26%**	+15%*
	relative	+33%**	+54%**	+35%**	+37%**	+13%*
Uterus	absolute	-27%**	-41%**	-59%**	-49%**	N
	relative	-21%*	-37%**	-55%**	-44%**	N
Small intestine (with contents)						
	absolute	N	N	N	+11%*	+11%**
	relative	N	+13%**	+23%**	+21%**	+10%**

Values in the table indicate percentage of change against the control mean (+: increase, -: decrease).
N: No remarkable changes
* (**): $p \leq 0.05$ (0.01) (significantly different from the control group)

Histopathology: Main histopathological findings considered directly related to treatment were noted in the adrenal, intestine, stomach, kidney, uterus and vagina (Table 13). Minimal vacuolation of the fascicular cortical cells of the adrenal was observed in both

sexes in the OM:HCTZ:AML and OM:HCTZ groups, and in males in the amlodipine group. Additionally, minimal hypertrophy of adrenal glomerular cortical cells was noted in most animals receiving amlodipine alone. Both these findings were absent in the control group. In the intestine, diffuse mucosal thickening was observed in the cecum and colon in several males and females dosed with CS-8635 at 30/18.75/20 or 100:62.5:20 mg/kg/day and in the colon and rectum in a few males and/or females at 100:62.5:20 or 30:18.75:20 mg/kg/day in a non-dose-dependent manner. This finding was also noted in both sexes in the AML group and nearly absent in OM:HCTZ and control groups. In the stomach, submucosal fibrosis in the pylorus was observed in a few males dosed at 30:18.75:20 or 100:62.5:20 (OM:HCTZ:AML) mg/kg/day and in some females in all CS-8635 groups without dose-relationships. It was absent in other groups including the control group. This finding in the stomach was suggested to be related to ulcer that has been reported to be associated with administration of angiotensin-II receptor antagonist; therefore, it was considered not to be a new emerging toxicity by the triple combination.

Table 13. Treatment-related microscopic findings for scheduled sacrifices

Test group		Control		OM/HCTZ		OM/HCTZ/AML						AML	
OM/HCTZ/AML Dose (mg/kg/day)		0/0/0		100/62.5/0		100/62.5/10		100/62.5/20		30/18.75/20		0/0/20	
Sex		M	F	M	F	M	F	M	F	M	F	M	F
No. of animals		15	15	15*	15	15*	15	15	15	15	15	15	15
Adrenal													
Vacuolation, cortical cell, fascicular	(±)	0	0	1	1	5	1	7	2	11	4	2	0
Hypertrophy, cortical cell, glomerular	(±)	0	0	0	0	0	0	0	0	0	0	12	11
Intestine, cecum													
Thickening, mucosal, diffuse	(±)	2	0	0	1	2	0	6	3	5	3	7	9
Intestine, colon													
Thickening, mucosal, diffuse	(±)	0	0	0	0	0	0	2	1	1	1	2	0
Intestine, rectum													
Thickening, mucosal, diffuse	(±)	0	0	0	0	0	0	0	1	2	0	1	1
Kidney													
Mineralization, papillary	(±)	2	3	5	6	6	6	5	4	10	9	2	9
Regeneration, tubular	(±/+)	0	0	14	3	15	5	13	7	14	5	0	0
Thickening, arterial wall, afferent arterial wall/interlobular artery	(±/+)	0	0	15	14	15	13	15	12	15	15	0	0
Mammary gland													
Hypertrophy, ductal	(±)	0	0	0	0	0	0	0	2	0	0	0	0
Ovary													
Vacuolation, lutein cell	(±/+)	/	0	/	15	/	15	/	15	/	15	/	0
Stomach													
Fibrosis, submucosal, pyloric	(±/+)	0	0	0	0	0	4	2	1	1	3	0	0
Spleen													
Hematopoiesis, extramedullary	(±)	15	3	12	4	8	0	1	0	3	2	15	3
Uterus													
Atrophy	(±/+)	/	0	/	3	/	6	/	12	/	8	/	2
Vagina													
Atrophy	(±/+)	/	0	/	5	/	4	/	12	/	9	/	5

*: Including one male each that died.
Values in the table indicate the number of animals with respective lesions.
M: Male, F: Female
/: Not applicable.
±: Minimal, +: Mild, ++: Moderate

In kidneys, a non-dose-dependent increased incidence (relative to control) of minimal or mild thickening of the arterial wall of the afferent arterioles/interlobular arteries and increased incidence of regeneration of renal tubules were noted in both sexes given OM:HCTZ:AML and OM:HCTZ combinations. These findings were absent in control and amlodipine groups and thus were attributable to OM:HCTZ treatment.

In females, minimal or mild hypertrophy of the mammary gland ducts was noted in 2 females receiving the high dose combination. Atrophy of the uterus and vagina, and vacuolation of the lutein cells in the ovary were noted in all CS-8635 groups without dose-relationships and in the OM:HCTZ group. A few animals in the amlodipine group displayed atrophy of the uterus and vagina. All of these findings were absent in the control group.

Toxicokinetics: Based on dose normalized AUC values in the combination groups, exposures to olmesartan (the active metabolite of OM), HCTZ and amlodipine increased with increase in dose but were not dose proportional. Systemic exposure to amlodipine, HCTZ and olmesartan increased with repetitive dosing, suggesting a tendency for accumulation. However, levels in week 4 and week 13 were comparable suggesting a steady state level reached in week 4 (Table 14). Both Cmax and AUC values for olmesartan and HCTZ were frequently higher (but not statistically significant) for males than for females but the values were similar for amlodipine in males and females.

There is a subtle difference in the accumulation of olmesartan or HCTZ as compared to amlodipine with repetitive dosing in the combination groups. The levels of olmesartan and HCTZ but not amlodipine were significantly increased in the combination groups (OM:HCTZ:AMLor OM:HCTZ) in weeks 4 and 13 relative to groups that received amlodipine alone. The differences in exposure to olmesartan between the high dose combination groups 100:62.5:10 or 100:62.5:20 (OM:HCTZ:AML) mg/kg/day and 100:62.5 (OM:HCTZ) mg/kg/day group for males were 3.4-fold and 5.0-fold (average for both groups) for Cmax and $AUC_{0\text{-}24h}$ values, respectively, for the week 13 measurement. The differences in exposure for females were 1.7-fold and 2.1-fold (average for both groups) for Cmax and $AUC_{0\text{-}24h}$ values, respectively, for the week 13 measurement. Similarly, the differences in exposure to HCTZ between the high dose combination groups 100:62.5:10 or 100:62.5:20 (OM:HCTZ:AML) mg/kg/day and 100:62.5 (OM:HCTZ) mg/kg/day group for males were 2.2-fold and 4.0-fold (average for both groups) for Cmax and $AUC_{0\text{-}24h}$ values, respectively, for the week 13 measurement. The differences in exposure for females were 1.5-fold and 2.1-fold (average for both groups) for Cmax and $AUC_{0\text{-}24h}$ values, respectively, for the week 13 measurement (Table 15). These results suggest that amlodipine augments the plasma concentration of olmesartan and HCTZ. By contrast, neither OM nor HCTZ had any effect on the amlodipine toxicokinetic parameters, although a significant accumulation was noted for amlodipine over the course of the study (week 13/day 1 ratio for Cmax 2.4, $AUC_{0\text{-}24h}$ 2.8 (average values for both males and females)).

Table 14. Mean toxicokinetic parameters for olmesartan (RNH-6270), HCTZ and amlodipine in rat plasma

Sex	Male				Female			
Test group	OM/HCTZ	OM/HCTZ/AML			OM/HCTZ	OM/HCTZ/AML		
OM/HCTZ/AML Dose (mg/kg/day)	100/62.5/0	100/62.5/10	100/62.5/20	30/18.75/20	100/62.5/0	100/62.5/10	100/62.5/20	30/18.75/20
No. of animals	5	5	5	5	5	5	5	5
RNH-6270								
C_{max} (ng/mL)								
Day 1	2910	2590	2070	1490	1770	1900	2120	1230
Week 4	2580	5840	7160	2480	2720	3690	10400	2180
Week 13	2340	8630	7240	5480	2950	4960	5050	2310
t_{max} (h)								
Day 1	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Week 4	2.0	2.0	2.0	2.4	2.0	2.0	2.0	2.0
Week 13	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
AUC_{0-24h} (ng·h/mL)								
Day 1	12200	14400	12700	7660	8000	11200	12000	5780
Week 4	15200	40300	66100	19500	18300	26100	83200	23300
Week 13	11600	48700	65800	25300	15600	31700	34800	12500
HCTZ								
C_{max} (ng/mL)								
Day 1	3200	5490	8110	3110	3030	4200	8540	3000
Week 4	6410	7600	7800	1620	5860	6090	6930	1630
Week 13	4470	10800	8950	2220	5210	8220	7440	1940
t_{max} (h)								
Day 1	2.0	2.8	4.0	4.0	2.0	2.8	3.2	3.2
Week 4	4.0	4.4	3.2	4.0	2.4	3.4	2.4	4.4
Week 13	2.0	2.4	2.8	2.0	2.0	2.0	2.0	2.0
AUC_{0-24h} (ng·h/mL)								
Day 1	22700	40600	63900	28500	16700	30300	59900	22600
Week 4	72600	90900	94900	17800	53700	58600	71900	19000
Week 13	27800	112000	108000	17100	27800	57800	61100	13100

Sex	Male				Female			
Test group	OM/HCTZ/AML			AML	OM/HCTZ/AML			AML
OM/HCTZ/AML Dose (mg/kg/day)	100/62.5/10	100/62.5/20	30/18.75/20	0/0/20	100/62.5/10	100/62.5/20	30/18.75/20	0/0/20
No. of animals	5	5	5	5	5	5	5	5
Amlodipine								
C_{max} (ng/mL)								
Day 1	52.6	158	154	167	57.4	171	160	169
Week 4	72.5	323	230	335	71.9	223	271	326
Week 13	137	359	357	411	101	251	309	364
t_{max} (h)								
Day 1	2.4	2.0	2.0	6.0	2.0	2.0	2.0	2.0
Week 4	4.8	4.6	5.4	7.0	3.2	5.8	6.0	3.4
Week 13	5.8	6.0	4.8	2.4	3.0	3.4	2.4	3.4
AUC_{0-24h} (ng·h/mL)								
Day 1	606	1810	2010	2540	637	1900	1960	2150
Week 4	1090	4830	3440	5360	953	3420	3950	5110
Week 13	2090	5870	5440	6520	1390	3650	4640	6350

Value in the table indicates the mean value.

Table 15. Effect of amlodipine on olmesartan and HCTZ exposure in rats co-administered amlodipine, OM and HCTZ for the week 13 measurement

Test group	Amlodipine mg/kg/day	Differences in exposure to olmesartan in the absence and presence of AML		Differences in exposure to HCTZ in the absence and presence of AML	
		M	F	M	F
OM/HCTZ	0	1.0	1.0	1.0	1.0
OM/HCTZ/AML	10	4.2	2.0	4.0	2.1
	20	5.7	2.2	3.9	2.2

7. Integrated Summary and Safety Evaluation

CS-8635 (Tribenzor®) is a fixed dose combination of olmesartan medoxomil (OM), hydrochlorothiazide (HCTZ) and amlodipine besylate (AML). Olmesartan medoxomil is a non-peptidic, orally effective, specific antagonist of angiotensin II, binding to the AT-1 receptor. It was developed by Daiichi Sankyo and was approved in April 2002 for the treatment of essential hypertension (Benicar®, NDA 21,286). HCTZ, a diuretic, was approved for the treatment of hypertension in 1959. Racemic amlodipine is a dihydropyridine calcium channel antagonist. Developed by Pfizer, it was approved in 1992 as the besylate salt for the treatment of hypertension, chronic stable angina and vasospastic angina (Norvasc®, NDA 19,787). The combinations of OM and HCTZ (Benicar HCT®), and OM and AML (Azor®) were approved in 2002 and 2007, respectively.

A combination of these three drugs is expected to result in an additive or synergistic antihypertensive effect when compared to single drug treatment. The only nonclinical GLP study performed with a OM:HCTZ:AML is a single 3 month toxicity study in rats. In this study, the drugs were administered at doses of 30:18.75:20, 100:62.5:10, 100:62.5:20 (OM/HCTZ/AML) mg/kg/day. Two additional groups of rats received 100:62.5:0 (OM/HCTZ/AML) mg/kg/day or 20 mg amlodipine/kg/day.

The oral administration of OM (100 mg/kg/day) and HCTZ (62.5 mg/kg/day) either with or without AML (10 mg/kg/day) to F344 rats for 3 months resulted in death of 2 males, none of which were attributed to drug treatment. A statistically significant and non-dose-dependent decrease in mean body weight gain relative to concurrent control was noted for both sexes with all dose combinations for the entire duration of the study. Mean weekly food consumption was significantly ($p < 0.05$) and non-dose-dependently reduced relative to concurrent control in the triple combination groups and the reduction was more severe for males (entire duration of the study) than females (until study day 43). Non-dose-dependent, statistically significant decreases in erythroid parameters including reticulocytes relative to control were noted for both sexes at all combination doses. A treatment-related, dose-dependent increase ($p < 0.05$) in blood urea nitrogen (up to 2.7-fold) was observed in both sexes with all dose combinations. Mild, dose-dependent increase ($p < 0.05$) in creatinine and potassium was noted in all dose groups except for amlodipine alone. However, changes in BUN, creatinine and potassium were intensified by co-administration with AML in males. Relative (but not absolute) kidney weights were statistically significantly increased in all combination groups.

Histopathologically, in the kidneys of males and females at all combination doses, a non-dose-dependent increased incidence of thickening of the arterial wall of the afferent arterioles/interlobular arteries and regeneration of renal tubules were noted. These findings were absent in control and amlodipine groups and thus were attributable to OM and HCTZ treatment. In the stomach, submucosal fibrosis in the pylorus was observed in a few males dosed at 30:18.75:20 or 100:62.5:20 (OM:HCTZ:AML) mg/kg/day and in some females in all CS-8635 groups without dose-relationships, but was absent in other groups including control. Also, statistically significant and dose-dependent increases in mean absolute and relative stomach weights were noted for both sexes with all dose combinations relative to control. This finding in the stomach was suggested

to be related to ulcer that has been reported to be associated with administration of angiotensin II receptor antagonist; therefore, it was considered by the sponsor not to be a new emerging toxicity by the triple combination. Absence of this finding in both OM:HCTZ and amlodipine alone groups suggests that amlodipine might potentiate the effect of OM. In the intestine, diffuse mucosal thickening was observed in the cecum and colon in several males and females dosed with CS-8635 at 30:18.75:20 or 100:62.5:20 mg/kg/day and in the colon and rectum in a few males and/or females at 100:62.5:20 or 30:18.75:20 mg/kg/day in a non-dose-dependent manner. This finding was also noted in both sexes in the AML group and nearly absent in OM:HCTZ and control groups. It was attributed to AML and OM treatment. Prolonged retention of intra-intestinal contents was suggested to involve in the findings due to relaxation of the intestinal smooth muscle by AML. Minimal vacuolation of the fascicular cortical cells of the adrenal was observed in both sexes in the OM:HCTZ:AML and OM:HCTZ groups, and in males in the amlodipine group. The incidence of vacuolation was slightly greater in the triple combination groups than the other groups, especially in males; however, the severity was comparable to that of the other groups. Additionally, minimal hypertrophy of adrenal glomerular cortical cells was noted in most animals receiving amlodipine alone. Both these findings were absent in the control group. These findings in adrenal were attributed to AML and OM treatment.

In females, atrophy of the uterus and vagina, and vacuolation of the lutein cells in the ovary were noted in all combination groups without dose-relationships. A few animals in the amlodipine group displayed atrophy of the uterus and vagina. The incidence and severity were greater in the triple combination groups when compared to those in other groups. All of the above findings in the female were absent in the control group. These findings were supported by statistically significant and non-dose-dependent decreases in absolute and relative uterus weight (AML group excluded) and increases in ovary weights relative to control. A NOAEL for all of these effects has not been established.

In the toxicokinetics evaluation, OM is rapidly and completely bio-activated by ester hydrolysis to olmesartan (RNH-6270) during absorption from the gastrointestinal tract. Systemic exposure to olmesartan, HCTZ and amlodipine increased with repetitive dosing of OM, HCTZ and AML besylate, suggesting a tendency for accumulation in both males and females. There were no substantial gender differences for either Cmax or AUC values. There is a subtle difference in the accumulation of olmesartan and HCTZ in the presence of AML with repetitive dosing in the combination groups. The levels of olmesartan and HCTZ were significantly increased (1.7- to 5-fold) in the combination groups in weeks 4 and 13 relative to groups that did not receive AML. This suggests that AML besylate augments the plasma concentrations of olmesartan and HCTZ. Similar findings were previously reported for the approved NDA 22100 (Azor®, a fixed dose combination of OM and AML). The mechanistic studies conducted by the sponsor for that NDA suggested the influence of AML on the absorption of OM (and HCTZ) in the gut. This is due to delayed OM/HCTZ evacuation via the relaxation of the intestinal smooth muscle, a consequence of excessive pharmacological effects of AML. By contrast, OM and HCTZ had no effect on the amlodipine toxicokinetic parameters.

Evaluation

The target organs of toxicity in both sexes were the kidney (thickening of the arterial wall of the afferent arterioles/interlobular arteries and regeneration of tubules), stomach (submucosal fibrosis in the pylorus), intestine (diffuse mucosal thickening in the cecum and colon) and adrenal (vacuolation of the fascicular cortical cells). In females, target organs also include the ovary (vacuolation of the lutein cells), uterus (atrophy) and vagina (atrophy). These findings were also observed in previous studies with OM alone at 100 or more mg/kg/day for 3 months (see NDA 21,286) and combination of OM with HCTZ or AML (NDA 21,532 for OM/HCTZ; NDA 22,100 for OM/AML). The change in the kidney and erythroid parameters is considered to be associated with the combined administration of OM and HCTZ. It is well known that angiotensin II receptor antagonists and ACE inhibitors induce hyperplasia/hypertrophy of the juxtaglomerular cells, including thickening of the afferent arterioles, by stimulation of renin production. The observation of submucosal fibrosis in the pyloric region of the stomach in the triple combination groups suggested a healing process from ulcer. Ulceration in the glandular stomach has been reported for several angiotensin II receptor antagonists including OM. The incidence of mucosal thickening in the intestine was greater in the amlodipine group rather than in the CS-8635 groups. Prolonged retention of intra-intestinal contents due to relaxation of the intestinal smooth muscle by AML might have contributed to the overall effect of OM and HCTZ. Additional toxicities such as vacuolation of the fascicular cortical cells of the adrenal in both sexes, and atrophy of the uterus and vagina, and vacuolation of the lutein cells in the ovary in females, were noted in all combination groups. Except for the vacuolation of the lutein cells, all of these toxicities were also noted in rats given AML alone. Again, the incidence and severity of each of these toxicities were greater in the triple combination groups compared to those in other groups. It should be noted that the maximal dose of OM used in this study was <10% (100 mg/kg/day) of that used in studies with OM alone (1000 to 2000 mg/kg/day). Still we may conclude that both HCTZ and AML augment the toxicities OM. It is unlikely that a significant increase in systemic exposure to olmesartan (2- to 5-fold increase in AUC) and HCTZ (2- to 4-fold increase in AUC) noted in the presence of AML besylate was responsible for increased toxicities noted in all combination groups since a similar enhanced effects were also noted in the OM:HCTZ combination group. On the other hand, clinical pharmacology studies (CS8635-A-U101 and CS8635-A-U102) indicated no pharmacokinetic interactions between the active components of CS-8635. Therefore, the sponsor concludes that the observed increase in systemic exposure to olmesartan and HCTZ by co-administration with AML in rats has no significant relevance to the clinical situation.

A NOAEL for the toxicities noted above could not be established in rats. Exposure to olmesartan, HCTZ and AML at the lowest dose studied in rats was 3, 14.5 and 5.2 (based on mean AUC values for both sexes) times higher than the expected exposure at the maximum recommended human dose [40:25:10 mg (OM:HCTZ:amlodipine); Table 16]. In spite of this enhancement of toxicity and absence of NOAEL for kidney, gastrointestinal tract, adrenal gland pathology, and decrease in body weight gain and erythroid parameters, the target organ toxicities are monitorable, reversible and attributable to the effects of the individual components of the combination, which have been used, often concomitantly, to treat hypertensive patients since the approval of OM for this indication in 2002.

Table 16. Human CS-8635 exposure multiples in 3 month toxicity study in rats

Drug	NOAEL[a] (mg/kg/day)	Gender	$AUC_{0\text{-}24h}$ (ng·h/ml)[b]	C_{max} (ng/ml)[b]	Exposure multiples based on [c]	
					$AUC_{0\text{-}24h}$	C_{max}
OM	30	male	25300	5480	4.1	6.0
		female	12500	2310	2.0	2.5
HCTZ	18.75	male	17100	2220	16.4	13.7
		female	13100	1940	12.6	12.0
Amlodipine	10	male	2090	137	6.2	18.3
		female	1390	101	4.1	13.5

a: No-Observed-Adverse-Effect-Level, since a NOAEL was not established in this study, the lowest doses used in the study is given in this column;
b: week 13;
c: Since a NOAEL was not established in this study, the exposure multiples are expected to be less than those indicated in the table.
OM, HCTZ and amlodipine exposure multiples were based on the mean human $AUC_{0\text{-}t}$ = 6134, 1043, 339 ng·h/ml and C_{max} = 913, 162, 7.5 ng/ml, respectively, after single oral dose of 40:25:10 mg (OM:HCTZ:amlodipine) to male and female healthy subjects (Study #CS8635-A-U101)

Recommendations on Labeling: See page 4.

Application Type/Number	Submission Type/Number	Submitter Name	Product Name
NDA-200175	ORIG-1	DAIICHI SANKYO INC	CS-8635 Combination of olmesartan medoxomil/amlodipine/hydrochlorothiazide

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