CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

209360Orig1s000

NON-CLINICAL REVIEW(S)

Tertiary Pharmacology Review

By: Paul C. Brown, Ph.D., ODE Associate Director for Pharmacology and Toxicology, OND IO
NDA: 209360
Submission date: 6-29-2017
Drug: Angiotensin II (synthetic human-sequence 8 amino acid peptide)
Applicant: La Jolla Pharmaceutical Co.
Indication: increase blood pressure in distributive or vasodilatory shock

Reviewing Division: Division of Cardiovascular and Renal Products

Discussion:
The primary pharm/tox reviewer and supervisor concluded that the information submitted was adequate to support the approval of angiotensin II for the indication listed above.

Only limited nonclinical studies were conducted in support of this NDA. No toxicology, carcinogenicity or animal reproduction studies were conducted with the product. This is acceptable given the acute use of the product and because the active ingredient is an endogenous peptide.

Angiotensin II can be classified as a vasoconstrictor as its Established Pharmacologic Class.

Conclusions:
The pharmacology/toxicology reviewer conducted a thorough evaluation of the nonclinical information submitted in support of this NDA. I agree that this NDA may be approved for the above indications from a pharm/tox perspective. I have provided comments on labeling separately.

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/s/

PAUL C BROWN
12/14/2017

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:	209360
Supporting document/s:	0001 (6/29/17), 0005 (7/28/17), 0009 (9/8/17)
Applicant's letter date:	6-29-2017
CDER stamp date:	6-29-2017
Product:	Angiotensin II
Indication:	Treatment of hypotension in adults with distributive or vasodilatory shock who remain hypotensive despite fluid and vasopressor therapy
Applicant:	La Jolla Pharmaceutical company, San Diego, CA 92121
Review Division:	Cardiovascular and Renal Products
Reviewer:	G. Jagadeesh, Ph.D.
Supervisor/Team Leader:	Thomas Papoian, Ph.D.
Division Director:	Norman Stockbridge, M.D., Ph.D.
Project Manager:	Sabry Soukehal
Date of review submission	November 19, 2017

Disclaimer

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Any information or data necessary for approval of NDA 209360 that the sponsor does not own or have a written right to reference constitutes one of the following: (1) published literature, or (2) a prior FDA finding of safety or effectiveness for a listed drug, as reflected in the drug's approved labeling. Any data or information described or referenced below from reviews or publicly available summaries of a previously approved application is for descriptive purposes only and is not relied upon for approval of NDA 209360.

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1 Executive Summary

1.1 Introduction (and Clinical Rationale)

The catecholamine norepinephrine is considered by many to be the standard vasopressor to treat septic shock (1,2). However, high doses of catecholamines may contribute to organ dysfunction and mortality in sepsis (3,4). Additionally, the antidiuretic hormone vasopressin has been proposed in the treatment of septic hypotension to reduce the need for catecholamine vasopressors and their potential adverse effects. However, in a large multicenter trial (VASST), vasopressin failed to reduce mortality in septic shock (5). Even short durations of hypotension are associated with increased severe adverse events such as myocardial infarction, stroke, and acute kidney injury (6). According to the sponsor, the currently available vasopressor therapies only leverage these two systems (7). The third is the renin-angiotensin aldosterone system (RAAS), a gatekeeper of a homeostatic system that regulates blood pressure and fluid and electrolyte balance (19). Angiotensin II (Ang II), the principal hormone of this system, is a potent vasoconstrictor, and might be able to reverse the vasoplegia associated with septic shock (9). The sponsor asserts that Ang II complements existing therapies by leveraging the RAS. Patients with hypotension who do not respond to fluid or vasopressor therapy may benefit from this currently unapproved class of vasopressor. Since 1941 (8), Ang II has been administered to humans in studies of vascular resistance and hypertension and has been given to healthy subjects for up to 11 days (reviewed in reference #9). Several reports of clinical use of Ang II to treat severe hypotension in septic shock have been published since 1991 (6,7,9-12). Based on the literature search, 31,281 subjects in 1144 studies received Ang II (0.5 - 3,780 ng/kg/min) (9).

The current drug product, LJPC-501 (Ang II acetate), an octapeptide is a first-in-class vasopressor that activates the renin-angiotensin system. It is a synthetic human Ang II with the Ile5-angiotensin II amino acid sequence. The sequence of Ang II in most mammalian species including human, horse, pig and rat is Asp-Arg-Val-Tyr-Ile-His-Pro-Phe. However, bovine Ang II (known as Hypertensin® produced by Ciba-Geigy Pharmaceutical company) differs from the rest of the species in having valine residue at position 5 instead of isoleucine. Many clinical studies have been conducted with both forms of Ang II (reviewed by Busse at al. 2017, reference #9). Detailed clinical studies (efficacy and safety) comparing both forms of Ang II are lacking although Kono et al. (1985) (13) reported greater pressor activity with human form (Ile5-Ang II) than with the bovine form (Val5-Ang II). On the other hand, Boyd et al. (1972) (14) and Oelkers et al. (1974) (15) showed similar pressor and aldosterone stimulating activities with both forms of Ang II.

Hypertensin® was approved for treatment of shock and circulatory collapse by the FDA under NDA 12-791 and marketed by Ciba-Geigy, but which now no longer exists. Hypertensin® was administered at a dose of 3-10 µg/minute for up to several days, but was used for longer durations in several clinical studies. The sponsor claims that a

slight difference in amino acid sequence between the active pharmaceutical ingredient in Hypertensin® and their LJPC-501 product is not expected to result in differences in safety or efficacy. Furthermore, the sponsor has conducted comparative receptor binding and cellular functional studies demonstrating similar potency for bovine Ang II and LJPC-501 (human Ang II). Through this NDA submission, La Jolla Pharmaceutical company is seeking regulatory approval for LJPC-501 for the treatment of hypotension in adults with distributive or vasodilatory shock who remain hypotensive despite fluid and vasopressor therapy.

1.2 Brief Discussion of Nonclinical Findings

Ang II activated AT-1 receptor takes precedent over the AT-2 receptor in cardiovascular effects of Ang II (16). Thus, it is unlikely that low expression levels, and health and disease patterns of AT-2 receptors afford any protection against AT-1 receptor mediated harmful effects. Actions of Ang II extend beyond blood pressure increase. At physiological doses, Ang II impairs insulin signaling (i.e., results in insulin resistance) (17), increases oxidative stress, and promotes production of superoxide radicals that scavenge free nitric oxide. Elevation of Ang II 2- to 3-fold more than the physiological levels is associated with many cardiovascular diseases including hypertension, atherosclerosis, aortic aneurysms, acute coronary syndrome and myocardial infarction (20,21). Chronic Ang II exposure provokes vascular inflammation and thrombosis. This is more apparent in pregnancy with pre-eclampsia (see below).

The toxicities of exogenously administered Ang II are manifested as a result of excessive pressor effects on the vasculature, heart and kidney, the target organs of action. The dose and duration of Ang II is critical in producing kidney and myocardial lesions, as has been observed in many publications. Single, continuous or repeat doses of intravenous administration of Ang II in rats and rabbits induced multifocal myocardial necrosis, together with renal failure and renal tubular necrosis. The severity of the renal and cardiac lesions is closely correlated with pressor effects and the elevation (2- to 3-fold normal values) of arterial plasma Ang II levels. A close interrelationship appears to exist between both the pressor and cytotoxic effects of Ang II and nitric oxide blockade. Blockade of nitric oxide synthesis and production during Ang II infusion causes blood pressure to increase more rapidly (additive effect) and results in rapid and extensive damage to the heart and kidneys. A preponderance of mortality and cardiovascular toxicity (coronary plaque erosion, thrombosis and subsequent myocardial infarction) was reported in hyperlipidemic rabbits with chronic infusion of angiotensin.

Several published in vitro and in vivo studies have demonstrated a genotoxic potential of Ang II. It causes DNA single and double strand breaks in abasic sites and increases the abundance of the DNA base modification, 7,8-dihydro-8-oxo-guanine. In experimental hypertension induced by infusion of Ang II, DNA lesions could be found in kidneys and heart of animals. The underlying mechanism is the AT-1 receptor activation

of reactive oxygen species generating enzymes like NADPH oxidase leading to oxidative stress.

Ang II has no adverse effect on male or female reproductive organs, fertility, organogenesis, or fetus development, survival and birth. Ang II thru AT-2 receptors induces ovulation and meiotic maturation of oocytes and favors a higher rate of embryo development. Increased Ang II during pregnancy raises blood pressure in the offspring, salt sensitivity is decreased and has a long-term impact on the offspring health. A correlation exists between a positive Ang II pressor response and early labor, fetal distress, and low birth weight.

In pre-eclampsia, elevated plasminogen activator inhibitor-1 occurs in the maternal circulation as circulating Ang II levels are highest, and this has been implicated as a contributing risk factor for hypercoagulation and fibrinolytic imbalance. Women with preeclampsia become hyper-responsive to the pressor effects of Ang II in late pregnancy and postpartum. Ang II responsiveness may remain elevated in this group, particularly among women with low sodium balance, reduced plasma volume and/or evidence of other underlying disorders. Pressor sensitivity to exogenous Ang II is decreased in normal pregnancy, but restored postpartum.

1.3 Recommendations

1.3.1 Approvability

Yes

1.3.2 Additional Non-Clinical Recommendations

Actions of Ang II extend beyond blood pressure increases. Continuous administration of Ang II for a long period provokes vascular inflammation and thrombosis. The latter is more pronounced in preeclampsia. The severity of the renal and cardiac lesions is closely correlated to pressor effects and the elevation (2- to 3-fold normal physiological range) of arterial plasma Ang II levels. A close interrelationship also exists between both the pressor and cytotoxic effects of Ang II and are potentiated with the co-administration of drugs that block nitric oxide synthesis and production. Excessive Ang II causes DNA single and double strand breaks. Therefore, it is recommended that treatment durations be kept to a minimum.

1.3.3 Labeling

Recommendations and edits are made on the labeling document on SharePoint and presented at the Division labeling meetings.

2 Drug Information

2.1 Drug

CAS Registry Number: 68521-88-0 (acetate salt)
4474-91-3 (free base)

Generic Name: Angiotensin II, human

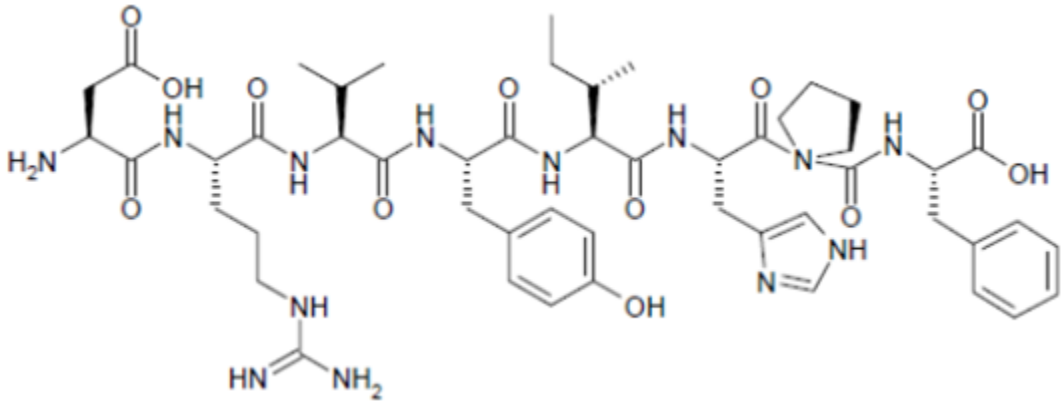
Code Name: LJPC-501, (b) (4)

Chemical Name: L-aspartyl-L-arginyl-L-valyl-L-tyrosyl-L-isoleucyl-L-histidyl-L-prolyl-L-phenylalanine, acetate salt

Molecular Formula/Molecular Weight: $C_{50}H_{71}N_{13}O_{12}$, 1045.5 gm/mol

Structure and Biochemical Description: LJPC-501 is a synthetic peptide hormone that is soluble in 0.9% sodium chloride solution. It is white to off-white powder, (b) (4) and freely soluble in water. Angiotensin II acetate is reversibly hygroscopic. There is a total of nine stereo-centers in the molecule; eight stereo-centers are in the peptide backbone. The remaining one is on the side chain of the isoleucine residue.

H-Asp1-Arg-Val-Tyr-Ile5-His-Pro-Phe8-OH



Pharmacologic Class: Vasopressor

2.2 Relevant INDs, NDAs, BLAs and DMFs

INDs 122708; (b) (4)

2.3 Drug Formulation

LJPC-501 drug product is a sterile, aqueous solution containing 2.5 mg/ml angiotensin II and 25 mg/ml mannitol in water, adjusted to pH 5.5 and is supplied in single-use vials. The drug product is supplied in two strengths: 2.5 mg angiotensin II/vial (2.5 mg/mL in 1 ml) and 5 mg angiotensin II/vial (2.5 mg/mL in 2 ml). It is diluted in 0.9% (v/v) sodium chloride solution prior to administration by intravenous infusion and is titrated to effect.

2.4 Comments on Novel Excipients

There are no novel excipients. The excipients used in the manufacture of the drug product include mannitol USP/Ph. Eur. (25 mg/ml) and water for injection USP/Ph. Eur. (b) (4) sodium hydroxide NF/Ph. Eur. and hydrochloric acid NF/Ph. Eur. are added, as needed, to adjust the pH.

2.5 Comments on Impurities/Degradants of Concern

None

2.6 Proposed Clinical Population and Dosing Regimen

Adults with distributive or vasodilatory shock who remain hypotensive despite fluid and vasopressor therapy.

The drug is given intravenously at 20 ng/kg/min. Titrated as frequently as every five minutes by increments of (b) (4) ng/kg/min as needed. During the first 3 hours, the maximum dose should not exceed 80 ng/kg/min. Maintenance dose should not exceed 40 ng/kg/min.

2.7 Regulatory Background

During the End of Phase 2 meeting discussions (August 16, 2016 meeting minutes Reference ID: 3983552), the Agency requested that bridging justification include comparative receptor binding and/or cellular functional studies demonstrating similar potency in those cases where the administered product cited in the literature differs in amino acid sequence from LJPC-501 drug product. Specifically, the Agency requested that La Jolla assess different forms of angiotensin II and their receptor pharmacology via receptor binding assays and/or functional activity assays for both angiotensin II receptor subtypes, angiotensin II receptor type 1 (AT1) and angiotensin II receptor type 2 (AT2). In addition, the Agency requested that La Jolla assess functional activity using two functional assays for each of the AT1 and AT2 receptor subtypes.

3 Studies Not Reviewed

None

4 Pharmacology

4.1 Primary Pharmacology

4.1.1. *In vitro studies*

4.1.1.1 Functional activity of LJPC-501 and analogs in CHO-K1 cells expressing the human Angiotensin II type 1 receptor

This non-GLP study (#R0182) was conducted at La Jolla Pharmaceutical Company, 10182 Telesis Court, 6th Floor, San Diego, CA. Study dates are not given. The objective of the study was to determine the functional activity of the AT1 receptor in Chinese hamster ovary cells using cyclic AMP and phosphorylated extracellular signal-regulated kinase (pERK) readouts after cellular stimulation in the presence of human forms of angiotensin II.

Methods

Three different forms of Ang II and an AT1 receptor antagonist, ZD7155, were used in the study (Table 1). Hypertensin®, Asn1-Val5-angiotensin II amide, manufactured by Ciba-Geigy Pharmaceutical Co., was not used in the study since it is no longer available.

Table 1. Compounds tested

Test Article (analog)	Abbreviations in Figures	Lot No.	Manufacture
Human angiotensin II acetate (Asp^1-Arg-Val-Tyr-**Ile**^{5}-His-Pro-Phe^8)	Human Ang II	Lot 1061913	(b) (4)
Bovine angiotensin II (Asp^1-Arg-Val-Tyr-**Val**^{5}-His-Pro-Phe^8)	Bovine Ang II	Lot 1052957	
LJPC-501 (Asp^1-Arg-Val-Tyr-**Ile**^{5}-His-Pro-Phe^8)	LJPC-501	Lot 2451-103	
ZD 7155	ZD 7155	Lot 5A/179629	

Human Ang AT1 receptors expressed in CHO cells were tested employing two downstream effectors of GPCR, cAMP and pERK (phophorylated extracellular signal-regulated kinase), after stimulation with three Ang II analogs listed in Table 1. In addition, a selective AT1 receptor antagonist, ZD7155, was used to demonstrate no activity or blockade of AT1 receptor stimulation. Although stimulation of AT-1 receptors (through Gi) inhibits adenylyl cyclase (AC) in several Ang II target tissues, it also modestly increases cAMP production in some target tissues (reviewed in Balakumar

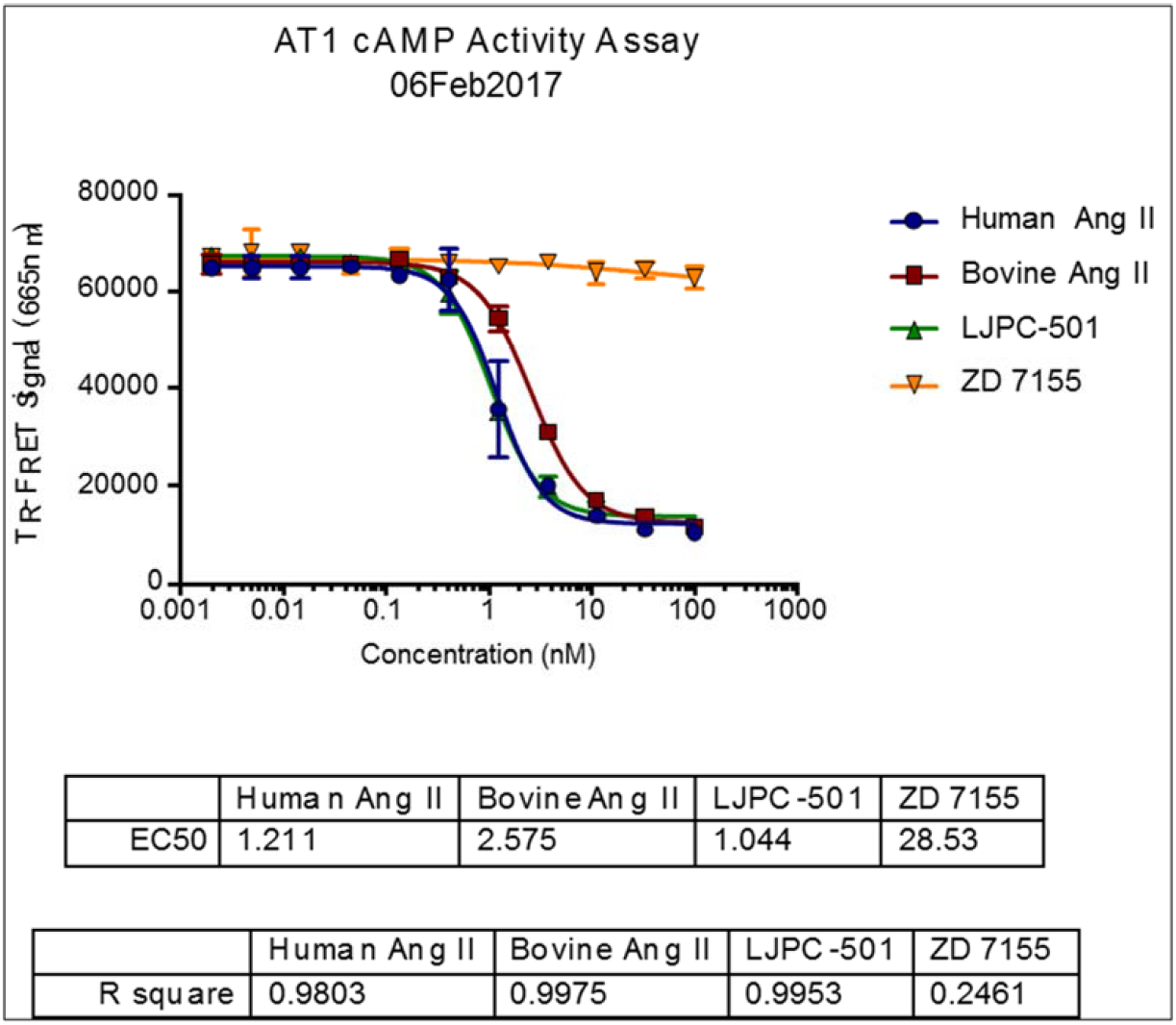
and Jagadeesh, 2014, reference #19). This results from the activation of calcium-sensitive AC. The sponsor initially tested for the inhibition of cAMP in the presence of Ang II. Later they determined that cAMP was being induced with the addition of Ang II in the selected cell line.

Results

Assays were performed on two different days by one analyst. The coefficient of variation between days of run was very high for both effectors assay. LJPC-501, human and bovine Ang II induced similar cAMP or pERK activity in the presence of angiotensin II in cells expressing the AT1 receptor. The mean EC50 values were not statistically significantly different among three forms of Ang II, although the mean values for bovine Ang II was somewhat different from either (b) (4) product or LJPC-501(Table 2, Fig. 1 and 2). A dose-dependent effect was clearly seen for all 3 forms of Ang II in both assays (Fig. 1 and 2). ZD 7155, a potent and selective competitive antagonist for the AT1 receptor did not elicit any response in either assay.

Table 2. cAMP and pERK mean EC50 Values (nM) in the human AT1 receptor transfected CHO-K1 cell line

Date of assay	**Human Ang II** (Ile^5-angiotensin II)	**Bovine Ang II** (Val^5-angiotensin II)	**LJPC-501** (Ile^5-angiotensin II)	**ZD 7155**
cAMP assay				
6-Feb-17	1.21	2.58	1.04	NA
8-Feb-17	0.823	1.54	0.847	NA
Mean	**1.02**	**2.06**	**0.946**	NA
pERK assay				
7-Feb-17	3.49	2.42	1.87	NA
9-Feb-17	4.53	5.40	3.46	NA
Mean	**4.01**	**3.91**	**2.66**	NA

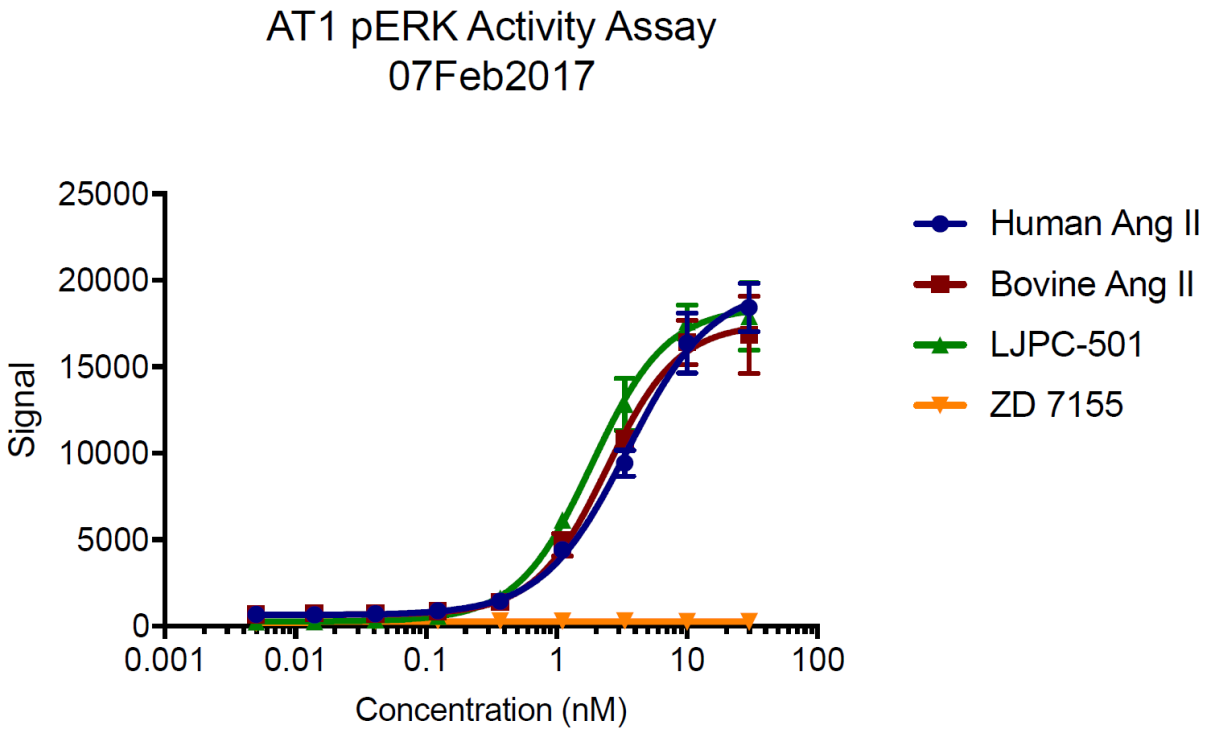


	Human Ang II	Bovine Ang II	LJPC-501	ZD 7155
EC50	1.211	2.575	1.044	28.53

	Human Ang II	Bovine Ang II	LJPC-501	ZD 7155
R square	0.9803	0.9975	0.9953	0.2461

TR-FRET Signal = the emission of light at 665 nm

Figure 1. AT1 cAMP dose response curve



	Human Ang II	Bovine Ang II	LJPC-501	ZD 7155
EC50	3.488	2.422	1.869	~ 0.01336

	Human Ang II	Bovine Ang II	LJPC-501	ZD 7155
R square	0.9898	0.9868	0.9897	0.4629

Signal = the emission of light at 520-620 nm

Figure 2. AT1 pERK dose response curve

4.1.1.2. Angiotensin II type 2 receptor radioligand binding assay

This non-GLP study (#R0207) was conducted at La Jolla Pharmaceutical Company, 10182 Telesis Court, 6th Floor, San Diego, CA. Actual studies were conducted at (b) (4). Study dates are not given. The objective of the study was to determine the affinity of bovine and human forms of Ang II to the human AT-2 receptor in Chinese hamster ovary cells.

Methods

Three different forms of Ang II and a selective AT-2 receptor agonist, Saralasin (sar1, ile8-ang II), were used in the study (Table 3). Hypertensin®, Asn1-Val5-angiotensin II amide, manufactured by Ciba-Geigy Pharmaceutical Co, was not used in the study since it is no longer available.

Table 3. Compounds tested

Test Article (analog)	Abbreviations in Figures	Batch/Lot No.	Manufacturer/Source
Human angiotensin II acetate (ile^5-angiotensin II)	Angiotensin II acetate salt	Lot 1061913	(b) (4)
Bovine angiotensin II (val^5-angiotensin II)	(Val5) – Angiotensin II	Lot 1052957	
LJPC-501 (ile^5-angiotensin II)	LJPC-501	Lot 2451-103	
Saralasin (sar^1, ile^8-angiotensin II)	Saralasin	Batch 403099	

Cell membrane preparations from CHO cells expressing human recombinant AT-2 receptors were incubated with a fixed concentration of radiolabeled an AT-2 receptor agonist ([125I] CGP-42112A). The bound radioligand was displaced by increasing concentrations of unlabeled test compounds. As the amount of unlabeled Ang II is increased, the amount of [125I] CGP-42112A bound to the receptor decreased. The IC_{50} that measures the affinity of the compound was calculated from the dose response curve.

Results

All three forms of Ang II displaced bound AT-2 receptor radioligand in a dose-dependent manner with high affinity (Fig. 3). The calculated IC_{50} values for the current investigative drug (LJPC-501) was approximately 2-fold lower (more potent, 0.248 nM) than (b) (4) human Ang II (0.558 nM) and (b) (4) bovine Ang II (0.506 nM) (Table 4). However, the sponsor contends that these results are within the expected degree of variability with

this assay. Saralasin, the selective AT-2 receptor agonist bound to AT-2 receptor with high affinity (IC_{50} 0.27 nM).

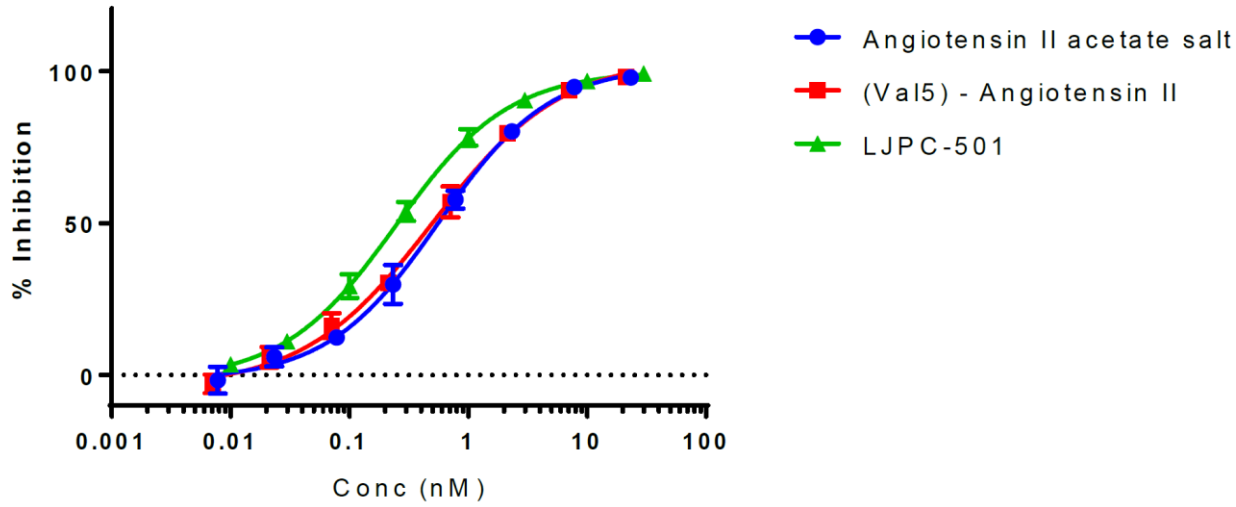


Figure 3. Radioligand binding % inhibition dose-response curve

Table 4. Calculated IC_{50} for each test compound

	Angiotensin II acetate salt (ile^5-angiotensin II) (nM)	(val^5-angiotensin II) (nM)	LJPC-501 (ile^5-angiotensin II) (nM)
Run 1	0.652	0.572	0.215
Run 2	0.509	0.501	0.284
Run 3	0.514	0.445	0.244
Mean	0.558	0.506	0.248
SD	0.081	0.063	0.035
%CV	14.5	12.5	14.1

SD = Standard deviation; %CV = percent coefficient of variance
Note: Each run was performed in triplicate (n=3). The means reflect the average of a total of 9 values generated over three separate assays.

4.1.2. *In vivo studies*

Results from the literature summarized in this section give an understanding of the pharmacologic properties of Ang II for the intended indication.

4.1.2.1 Comparison of fast and slow pressor effects of Ang II in the rat

Reference: Brown AJ et al. Comparison of fast and slow pressor effects of angiotensin II in the conscious rat. *Am. J. Physiol*. **241**:(Heart Circ. Physiol. 10):H381-H388 (1981).

Conscious female Wistar rats received either low (20 ng/kg/min for 7 days) or high (270 ng/kg/min for 1 hr) dose of Val5-Ang II. Control rats were infused with dextrose. Blood pressure and Ang II levels were measured in all rats during infusion.

Mean arterial blood pressure did not increase during the 1st hour infusion on day 1 of Ang at 20 ng/kg/min and the measured plasma concentration of Ang II was 89 pg/ml (approximately 2 times the control animals). However, on day 2, blood pressure was significantly increased by 15 mm Hg. Thereafter, it rose progressively daily, reaching a peak (49.7 mm Hg relative to dextrose control) on day 7 with plasma Ang II concentrations approximately 214 pg/ml, which was 6 times higher than that noted in control animals. On the other hand, in rats receiving Ang II at 270 ng/kg/min for 1 hour, a similar increase in bp (45.3 mm Hg) was observed with a 32-fold increase in plasma angiotensin II concentration.

The authors conclude that the slow pressor effect of Ang II develops at a plasma concentration of the peptide that is close to or within a few orders of magnitude to the physiological range. This slow pressor effect has little or no effect on food or water intake or on urinary excretion of sodium. Infusion of Ang II at low dose without direct pressor effect gradually raises blood pressure over 7 days to a level similar to the maximum direct pressor effect produced by larger doses of Ang II.

4.1.2.2 Effects of Ang II on regional afferent and efferent arteriole dimensions and the glomerular pole

Reference: Denton KM et al. Effects of angiotensin II on regional afferent and efferent arteriole dimensions and the glomerular pole. *Am J Physiol Regul Integr Comp Physiol*, **279** (2):R629-38 (2000).

The effect of exogenously administered Ang II on the pressor response in anesthetized rabbits was evaluated. Isoleu5-Ang II (human form) was administered intrarenally at doses of 0.1, 1 or 5 ng/kg/min, which corresponds to human equivalent doses of 0.032, 0.32, and 1.6 ng/kg/min for 20 min. Measurements of renal hemodynamics and function were made over a period of 1 hour. Renal vascular resistance, mean arterial pressure, renal blood flow and glomerular filtration rate were determined. After completing these measurements, kidneys were perfusion-fixed and vascular casts were made.

Diameters of interlobular, afferent, and efferent vessels from outer, midcortical, and juxtamedullary glomeruli were measured from scanning electron micrographs.

Ang II produced dose-dependent reductions in afferent and efferent arterioles luminal diameter in the outer, mid and inner cortex without affecting mean arterial pressure. The response was significantly ($P < 0.05$) greater in the juxtamedullary efferent arterioles than the outer or midcortical glomeruli. Renal blood flow decreased by 17, 35 and 65% relative to vehicle ($p < 0.005$) during infusion of 0.1, 1 and 5 ng/kg/min Ang II, respectively, with significant increases in renal vascular resistance ($p < 0.001$). Filtration fraction was 0.17 ± 0.01% in the vehicle group, rising to 0.20 ± 0.01, 0.27 ± 0.02, and 0.37 ± 0.03 in the 0.1, 1, and 5 ng/kg/min angiotensin II groups, respectively ($p < 0.001$).

In summary, intra-renal administration of Ang II in rabbits produced dose-dependent increases in renal vascular resistance, renal blood flow and filtration fractions, without affecting mean arterial pressure. A decrease in GFR was noted at the highest dose.

4.1.2.3 Effects of Ang II in experimental hypotensive hyperdynamic sepsis

Reference: Wan L, et al. Angiotensin II in experimental hyperdynamic sepsis. *Critical Care,* **13**:R190 (2009).

The objective of the study was to characterize the effects of Ang II during sepsis in sheep. In hyperdynamic sepsis there is a reduced renal blood flow (RBF), hypotension, systemic vasodilation and associated decrease in peripheral resistance. Experiments were conducted on conscious adult Merino ewes that received intravenous injection of live E. coli (3 × 109 colony forming units). After reaching the criteria for septic shock (8 to 12 hours after the initial injection of E. coli), animals were randomized to receive a 6-hr intravenous infusion of either Ang II (55 ± 78 ng/kg/min, range 4.25 to 450 ng/kg/min) or vehicle (saline). The study measured the systemic and regional hemodynamic effects and the renal functional effects of Ang II infusion compared with placebo.

Results: All animals developed features of sepsis. The onset of severe sepsis was associated with peripheral vasodilatation, hypotension and an increase in CO (hyperdynamic septic state). MAP decreased from 86.3 to 71.8 mmHg ($p < 0.0001$) and total peripheral conductance increased ($p < 0.0001$). These changes were accompanied by increases in CO and heart rate, and a reduction in stroke volume (67.5 to 46.4 mL/beat, $p < 0.0001$). Sepsis caused pronounced vasodilation in all regional vascular beds (increased renal conductance by 1.8 mL/min/mmHg, $p < 0.0001$ and increased RBF by 104 mL/min, $p < 0.0001$). Despite the increase in RBF there was a 46% decrease in urine output and a 43% decrease in creatinine clearance.

Intravenous infusion of angiotensin II increased and maintained MAP at baseline levels, while animals receiving vehicle remained hypotensive. The angiotensin II-induced increase in arterial pressure resulted from peripheral vasoconstriction with a small reduction in CO, but no significant effect on heart rate. Relative to placebo, Ang II

significantly reduced renal conductance and RBF to 3.3 mL/min/mmHg and 278.8 ± 86.0 mL/min (both $p < 0.0001$), respectively, which returned to levels similar to those in the pre-sepsis period (3.4 mL/min/mmHg and 292.3 mL/min, respectively, both $p > 0.05$). These effects were maintained for the 6-hour infusion, while in the vehicle group, renal conductance (5.2 ± 1.3 ml/min/mmHg) and RBF (358.7 ± 80.8 mL/min) remained elevated. Additionally, Ang II infusion increased urine output more than seven-fold (364.3 mL/h vs. 48.1 mL/h; $p < 0.0001$) relative to saline group. This effect was maintained throughout the experiment. Ang II also increased creatinine clearance to 80.6 mL/min, a value similar to pre-sepsis levels (88.7 mL/min, $p > 0.05$), while, in the vehicle-treated group, creatinine clearance remained low.

In summary, intravenous Ang II infusion restored arterial pressure and decreased renal blood flow while inducing a marked increase in urine output and normalizing creatinine clearance, indicating improved kidney function in an animal model of hypotensive hyperdynamic sepsis.

4.1.2.4 Regulation of Ang II receptors in septic shock

Reference: Schmidt C, et al. Blockade of multiple but not single cytokines abrogates downregulation of angiotensin II type-I receptors and anticipates septic shock. *Cytokine,* **49**:30–38 (2010).

Septic shock is characterized by systemic vasodilation with decreased reactivity to vasoconstrictors, leading to arterial hypotension, multiple organ dysfunction, and death. Additionally, an enhanced production of endogenous proinflammatory mediators such as TNF-α, IL-1β, IFN-γ, and IL-6 are involved in the regulation of vasoconstrictive receptors. This could account for diminished vascular reactivity in response to vasoconstrictors during sepsis. Patients with sepsis and septic shock often require escalating doses of vasoconstrictors to maintain blood pressure. The objective of the study was to characterize the effects of Ang II during sepsis in mice. The role of proinflammatory cytokines (TNF-α, IL-1β, IFN-γ and IL-6) in the pathogenesis of sepsis-induced circulatory failure with downregulation of angiotensin II type 1 receptors was evaluated.

Sepsis in wild-type mice and in mice with deficiencies for TNF-α, IL-1β, IFN-γ or IL-6 was induced by cecal ligation and puncture (CLP) and wild-type mice were injected with cytokines. Animals were treated with glucocorticoids or small interfering RNA (siRNA) targeting single or multiple cytokines or NF-κB. Vascular smooth muscle cells (VSMCs) were incubated with cytokines. Septic mice developed cardiovascular dysfunction as indicated by decreased systemic vascular resistance (SVR) and blood pressure, and increased cardiac index. These changes were associated with a significant liberation of proinflammatory cytokines, and a potent downregulation of AT1 receptor mRNA and protein. The latter finding likely explained the reduced blood pressure and SVR response to angiotensin II in septic animals compared to sham-treated mice. Injection of single proinflammatory cytokines also strongly downregulated AT1-receptors paralleled

by a markedly endogenous liberation of further cytokines, whereas, simultaneous blockade of these endogenously activated cytokines by dexamethasone prevented downregulation of AT1 receptors. Furthermore, inhibition of multiple but not single cytokines by treatment with siRNA against multiple cytokines or NF-κB significantly attenuated CLP-induced AT1 receptor downregulation and prevented septic circulatory failure. The data demonstrate that downregulation of AT1 receptors during sepsis is due to multiple, but no single cytokines, and define a relevant role for NF-κB in the pathogenesis of septic shock.

4.1.2.5 Renal bioenergetics during sepsis and Ang II infusion

Reference: May CN, et al. Renal bioenergetics during early Gram-negative mammalian sepsis and angiotensin II infusion. *Intensive Care Med,* **38**:886–893 (2012).

Sepsis is the most important cause of acute kidney injury (AKI) in critically ill patients accounting for approximately 50% of cases. Its incidence and mortality remain high, in part, because of our limited understanding of its pathogenesis. Somewhat paradoxically, intravenous infusion of Ang II appears to improve creatinine clearance and urinary output, while reducing the elevated RBF to control levels. Although a clinical biomarker of increased function, increased urinary output in early AKI may not be entirely desirable. In the setting of AKI, oxygen consumption for each unit of sodium reabsorbed is markedly increased, so that an increase in urinary output may increase renal oxygen consumption enough to induce bioenergetic failure (ATP depletion). Thus, the demonstration that renal ATP levels are unaffected by Ang II treatment is an important step in the assessment of the safety of vasoconstrictor therapy for septic AKI. Similarly, measurement of bioenergetics during mammalian sepsis would help clinicians understand how much global ischemia may or may not contribute to tubular injury in early sepsis. In the study, the authors measured renal ATP and RBF during hypotensive sepsis and during Ang II infusion. They tested the hypothesis that neither sepsis, nor Ang II infusion would induce renal ATP depletion.

Methods: Hypotensive sepsis was induced in 15 anesthetized adult Merino ewes by intravenous injection of live E. coli (3×10^9 colony forming units) over 5 minutes. Two hours after the E.coli injection, sheeps were randomly assigned to receive Ang II (10 - 600 ng/kg/min; N = 6) or vehicle (saline 12 mL/h; n = 5) by IV infusion. The dose of Ang II was titrated during the experiment to maintain mean arterial pressure (MAP) at the pre-sepsis control level. MAP, heart rate, RBF and renal ATP levels were measured. Renal vascular conductance (RVC) was calculated as RBF/MAP.

Results: Intravenous administration of E. coli caused a progressive reduction in MAP from 89 to 58 mmHg over 4 hours ($p < 0.001$) without any change in HR. Initially, renal vasodilatation with a significant increase in RVC and RBF was noted. After 4 hr, MAP and RBF decreased below control levels. Despite decreased RBF and hypotension, renal ATP was unchanged. Intravenous infusion of Ang II rapidly restored MAP to control levels (90 mmHg after 1 hour, and 96 mmHg after 2 hours), a value significantly

greater than in the control group ($p < 0.001$). It also caused renal vasoconstriction, but did not decrease RBF because of the associated increase in perfusion pressure. Ang II had no effect on the renal levels of total ATP.

In conclusion, infusion of Ang II restored MAP to baseline levels and significantly reduced RVC. Furthermore, there was no reduction in renal ATP levels. Thus, Ang II is not likely to cause kidney ischemia/damage during treatment of shock.

4.1.2.6 Ang II in septic shock in pigs

Reference: Corrêa TD, et al. Angiotensin II in Septic Shock: Effects on Tissue Perfusion, Organ Function, and Mitochondrial Respiration in a Porcine Model of Fecal Peritonitis. *Critical Care Med,* **42:**e550-559 (2014).

Norepinephrine is considered the standard vasopressor to treat septic shock. High doses of catecholamines may contribute to organ dysfunction and mortality in sepsis. Vasopressin has been proposed as an additional treatment of septic hypotension in order to reduce the need for catecholamine vasopressors and their potential adverse effects. However, vasopressin failed to reduce mortality in septic shock. Ang II, a strong physiologic vasoconstrictor, might be able to reverse the vasoplegia associated with septic shock. Some reports of its clinical use to treat severe hypotension in septic shock have been published. In short-term experimental sepsis, Ang II preserved renal function and bioenergetics despite reduction of renal blood flow. Ang II may modify tissue energy metabolism either directly or via its effects on tissue perfusion. Based on these observations, the authors have hypothesized that Ang II can be used to stabilize hemodynamics in experimental sepsis without adverse effects on renal function or mitochondrial respiration. To test this hypothesis, they compared the effects of Ang II and norepinephrine on systemic and regional hemodynamics, renal function, and renal, hepatic, and cardiac mitochondrial respiration in a clinically relevant porcine abdominal sepsis model.

Methods: Pigs were randomized to receive after 12 hours of fecal peritonitis fluid resuscitation either norepinephrine or Ang II (n = 8 each) for 48 hours. A separate group (n = 8), treated with enalapril for 1 week before peritonitis and until study end, received fluids and norepinephrine. The blood pressure dose-response to Ang II was evaluated in additional four nonseptic pigs.

Results: Exogenous Ang II infusion was as efficient as norepinephrine in maintaining arterial blood pressure and cardiac output. Exogenous angiotensin II did not increase the prevalence of AKI or deteriorate mitochondrial respiration. In the context of the resuscitation protocol, arterial blood pressure goals could not be achieved when angiotensin-converting enzyme was inhibited before and during sepsis. Creatinine concentrations increased despite maintained renal blood flow and urinary output, and renal mitochondrial respiration efficiency was increased with enalapril treatment. Thus, Ang II reversed sepsis-induced hypotension with systemic and regional hemodynamic effects similar to those of norepinephrine. Inhibition of angiotensin-converting enzyme

before sepsis worsened the hypotension, but enhanced skeletal muscle adenosine triphosphate.

4.1.2.7 Ang II and vasopressin in the defense system against anaphylactic hypotension

Reference: Wang M, et al. Angiotensin II and vasopressin are involved in the defense system against anaphylactic hypotension in anesthetized rats. *Eur J Pharmacol,* **731:** 38–43 (2014).

Anaphylactic shock is sometimes life-threatening, but the defense system against this circulatory failure was not fully understood. Ameliorating roles of ANG II and vasopressin in anaphylactic hypotension were investigated in anesthetized ovalbumin-sensitized Sprague-Dawley rats.
Methods: Rats were sensitized by the subcutaneous injection of an emulsion made by mixing equal volumes of complete Freund's adjuvant with ovalbumin in saline. Two weeks after injection, rats were randomly allocated to the following pretreatment groups (n = 7/group):
(1) Control (non-pretreatment), Saline – 500 µl IV injection
(2) ACE inhibitor- captopril, 2.5 mg/kg by a bolus injection followed by a continuous infusion (1 mg/kg/h) throughout the experimental period
(3) ARB- losartan, 6 mg/kg by IV injection
(4) V1a vasopressin receptor antagonist- [beta-mercapto-beta, beta-cyclopentamethylenepropionyl[1], O-me-Tyr2, Arg[8]]-vasopressin – 60 µg/kg by IV injection
Duration of Treatment: 1 day (bolus injection)
Anaphylactic shock was induced by an intravenous injection of antigen in sensitized animals. The systemic arterial pressure (SAP), central venous pressure (CVP), portal venous pressure (PVP) and portal venous blood flow (PBF) were measured, and splanchnic vascular resistance (Rspl: (SAP-PVP)/PBF) was determined.
Results: In the control group, an injection with antigen in sensitized animals caused systemic hypotension and portal hypotension; SAP decreased to a nadir of 33 mmHg at 15 minutes after injection followed by a gradual recovery towards baseline (95 mmHg at 120 minutes). Rspl decreased after antigen administration, but increased 15 minutes later to 1.5-fold and continued to increase to 1.9-fold (baseline) by 120 minutes. Pretreatment with losartan, captopril or V1a receptor antagonist augmented the initial decrease in SAP and attenuated the SAP recovery along with augmentation of the late increase in Rspl. Furthermore, in all pretreatment groups PVP increased in a manner similar to that in the control group, and no significant differences were found among sensitized groups. CVP and heart rate did not change significantly after antigen injection in any of the pretreatment groups. The 2-hour survival rate was significantly smaller in the pretreatment groups relative to the control group. Plasma levels of Ang II and vasopressin increased to 3.8- and 9.8-fold, respectively, at 30 minutes after antigen in the control group, whereas captopril pretreatment inhibited the increase in Ang II.
Conclusion: Inhibition of Ang II (by ACEi or ARB) or vasopressin exacerbates anaphylaxis-induced hypotension in anesthetized rats.

4.2 Secondary Pharmacology

4.2.1 Angiotensin II activates gluconeogenesis in rats

Reference: Coimbra C.C. et al. Gluconeogenesis activation after intravenous angiotensin II in freely moving rats. *Peptides,* **20**:823–827 (1999).

The renin-angiotensin system (RAS), besides affecting fluid volume, electrolytes and hemodynamic states, is also involved in the regulation of metabolic and endocrine functions, especially blood glucose homeostasis. This hyperglycemic effect of Ang II can be attributed to its direct action on hepatic glucose production in addition to its stimulatory action on the sympathoadrenal system. The direct hyperglycemic action of Ang II is presumably related to the peptide-induced increase of phosphorylase activity that enhances glycolysis and hepatic glucose output. Ang II also amplifies gluconeogenesis in vitro, probably stimulating pyruvate carboxylase and inhibiting pyruvate-kinase. However, the underlying biochemical mechanism for the increase in hepatic glucose output induced by IV injection of Ang II has not been hitherto investigated. This study was performed to explore the role of Ang II on hepatic glucose output (i.e., gluconeogenesis) in rats.

Methods: The glycemic action of Angiotensin II was evaluated in fed and 20-hour fasted rats. Blood samples were collected immediately before and 10 and 20 minutes after intravenous injection of Ang II (0.95 nmol/100 g body weight). All experiments were performed with rats freely moving in the cage. Gluconeogenesis was evaluated in fed rats. On the day of the experiment, trace amounts of [14C]-bicarbonate dissolved in bicarbonate-free Krebs–Ringer phosphate buffer, pH 7.4, were continuously infused at a rate of 60 µL/minute (0.18 mCi/min). Blood samples (0.3 mL) were collected at 30, 40, 45, 50, 55, and 60 minutes after the beginning of the infusion. Blood [14C]-glucose was isolated and its radioactivity determined. Blood glucose concentration was determined with glucose oxidase. Ang II or saline was injected IV immediately after withdrawal of the 40-minute blood sample.

Results: After IV injection of 0.15 M NaCl, plasma glucose levels of fed (N = 10) and fasted (N = 6) controls did not differ significantly from preinjection levels. In contrast, IV injection of Ang II in both fed (N = 12) and fasted rats (N =7) resulted in sharp and marked increases in plasma glucose concentration (9.3 mM at 10 minutes vs. 7.0 mM, basal for fed rats, and 6.5 mM at 10 minutes vs. 5.4 mM, basal for fasted rats). Although both groups showed significant hyperglycemic responses to Ang II injection, the fasted group showed lower ($p < 0.05$) increments in blood glucose levels when compared to the fed group (30.3% in fed rats vs. 20.4% in fasted rats).

In control animals, the incorporation of ^{14}C into circulating glucose increased slowly and progressively during [^{14}C]-bicarbonate infusion. The rate of increase was not significantly affected by IV injection of 0.15 M NaCl (n = 7). A similar rate was obtained initially in the Ang II treated group (N = 10). However, 15 minutes after Ang II injection the rate of ^{14}C incorporation into blood glucose increased abruptly, becoming

significantly higher ($p < 0.01$) than the rate observed in control rats, suggesting that IV injection of Ang II results in an activation of de novo glucose synthesis in fed rats. Despite this significant Ang-induced increase in the rate of ^{14}C incorporation into blood glucose, the increase was delayed when compared with the effect of Ang II on total plasma glucose concentration. Plasma glucose levels after Ang II injection were already significantly elevated after the first experimental interval (5 minutes, $p < 0.01$), whereas an increased rate of ^{14}C incorporation into blood glucose was first detected only at 15 minutes post-injection.

In conclusion, the hyperglycemia induced by IV administration of Ang II is accompanied by an activation of gluconeogenesis, as evidenced by a rapid and marked increase in the rate of incorporation of ^{14}C from [^{14}C]-bicarbonate into circulating glucose.

4.2.2 Angiotensin II–induced insulin resistance in rats

Reference: Ogihara, T. et al. Angiotensin II–Induced Insulin Resistance Is Associated with Enhanced Insulin Signaling. *Hypertension,* **40:**872-879 (2002).

Ang II is involved in the pathogenesis of both hypertension and insulin resistance, though few studies have examined the relationship between the two. Hypertensive individuals are more likely to become diabetic than normotensives. It is therefore notable that Ang II is reportedly involved in the development of both hypertension and insulin resistance, and agents that inhibit the action of Ang II (ACEi and ARBs), not only reduce blood pressure but also restore insulin sensitivity. It has been suggested that crosstalk between Ang II and insulin signaling pathways underlies Ang II-induced insulin resistance. The authors therefore investigated the effects of chronic Ang II infusion on blood pressure and insulin sensitivity in rats fed a normal or high-salt diet.

Methods: This study was performed on seven-week-old male Sprague-Dawley rats. They were intravenously infused with normal saline (control group) or Ile5-angiotensin II ((b) (4)) for 12 days at a rate of 100 ng/kg/minute. Ang II-infused rats were fed a standard rodent diet containing 0.3% NaCl (Ang II group) or high-salt diet containing 8% NaCl (Ang II + salt group) during the infusion period.

Results: AII infusion for 12 days significantly elevated blood pressure and significant insulin resistance, assessed by a hyperinsulinemic-euglycemic clamp study and glucose uptake into isolated muscle and adipocytes. High-salt loading exacerbated the effects of Ang II infusion significantly. Despite the insulin resistance, insulin-induced tyrosine phosphorylation of the insulin receptor and insulin receptor substrates, activation of phosphatidylinositol (PI) 3-kinase, and phosphorylation of Akt were all enhanced by Ang II infusion. Subsequently, to investigate whether oxidative stress induced by Ang II contributes to insulin resistance, the membrane-permeable superoxide dismutase mimetic, tempol, was administered to Ang II-infused rats. Chronic Ang II infusion induced an accumulated plasma cholesterylester hydroperoxide levels, indicating the increased oxidative stress, whereas the treatment with tempol normalized plasma cholesterylester hydroperoxide levels in Ang II-infused rats. In addition, the treatment

with tempol normalized insulin resistance in Ang II-infused rats, shown as a decreased glucose infusion rate in the hyperinsulinemic euglycemic clamp study and a decreased insulin-induced glucose uptake into isolated skeletal muscle, as well as enhanced insulin-induced PI 3-kinase activation to those in the control rats. These results strongly suggest that Ang II-induced insulin resistance cannot be attributed to impairment of early insulin-signaling steps, and that increased oxidative stress, possibly through impaired insulin signaling located downstream from PI 3-kinase activation, is involved in Ang II-induced insulin resistance.

4.3 Safety Pharmacology

4.2.1 Inhibition of the rapid component of the delayed rectifier potassium current by Ang II in rats

Reference: Wang, YH. et al. Inhibition of the rapid component of the delayed rectifier potassium current in ventricular myocytes by angiotensin II via the AT1 receptor. *British Journal of Pharmacology,* **154:**429–439, (2008).

Human ether-a-go-go-related gene (hERG or KCNH2) encodes the poreforming subunit of the channel underlying I_{Kr}, which is crucial for the repolarization of cardiac action potentials. A reduction in hERG currents due to either genetic defects, or adverse drug effects can lead to hereditary or acquired long QT syndrome in humans characterized by action potential prolongation, lengthening of the QT interval on the surface ECG, and an increased risk for 'torsade de pointes' ventricular arrhythmias and sudden cardiac death. I_{Kr} also represents a target for modulation by autonomic neurotransmitters and hormones. There is increasing evidence that Ang II is associated with the occurrence of ventricular arrhythmias. However, little is known about the electrophysiological effects of Ang II on ventricular repolarization. The rapid component of the delayed rectifier K^+ current (I_{Kr}) plays a critical role in cardiac repolarization. The information available is limited regarding the effect of Ang II on repolarizing K^+ currents and resultant changes in action potential duration (APD) in cardiac ventricular myocytes. The present study was designed to examine the possible regulation of I_{Kr} /hERG currents by Ang II in guinea-pig isolated ventricular myocytes and heterologous expression system using the whole-cell patch-clamp technique. These results provide direct evidence that Ang II produces an acute inhibitory effect on I_{Kr} /hERG currents via the AT1 receptor linked to the PKC pathway in ventricular myocytes.

Methods: Single ventricular myocytes were enzymatically dissociated from the heart of adult guinea-pigs and used for electrophysiological recording within 6-8 hours after isolation. The whole-cell patch-clamp technique was used to record I_{Kr} in native cardiocytes and in human embryonic kidney (HEK) 293 cells, co-transfected with hERG encoding the a-subunit of I_{Kr} and the human AT1 receptor gene. The APD was measured at 50% and 90% repolarization (APD50 and APD90, respectively). Duration of treatment with Ang II was 10 min.

Results: Effects on I_{Kr} in Guinea-Pig Ventricular Myocytes: Ang II markedly reduced the tail currents during repolarizations relative to control (untreated myocyte). The reduction of I_{Kr} tail current occurred within 2-3 minutes and reached saturation about 10 minutes after addition of Ang II (100 nM). The inhibition of I_{Kr} by Ang II was not reversible, and Ang II was significantly more potent at inhibiting the tail current at more positive potentials (Fig. 4). The tail current density of I_{Kr} was decreased from 0.54 ± 0.04 to 0.39 ± 0.05 pA/pF at a prepulse potential of 40mV (N = 6, $p < 0.01$). Ang II decreased the amplitude of I_{Kr} in a concentration-dependent manner with an IC50 of 8.9 nM. PKC inhibitors, staurosporine (100 nM) and Bis-1 (300 nM), significantly attenuated Ang II-induced inhibition of IKr. Losartan (1 μM) abolished Ang II-induced inhibition of the IKr.

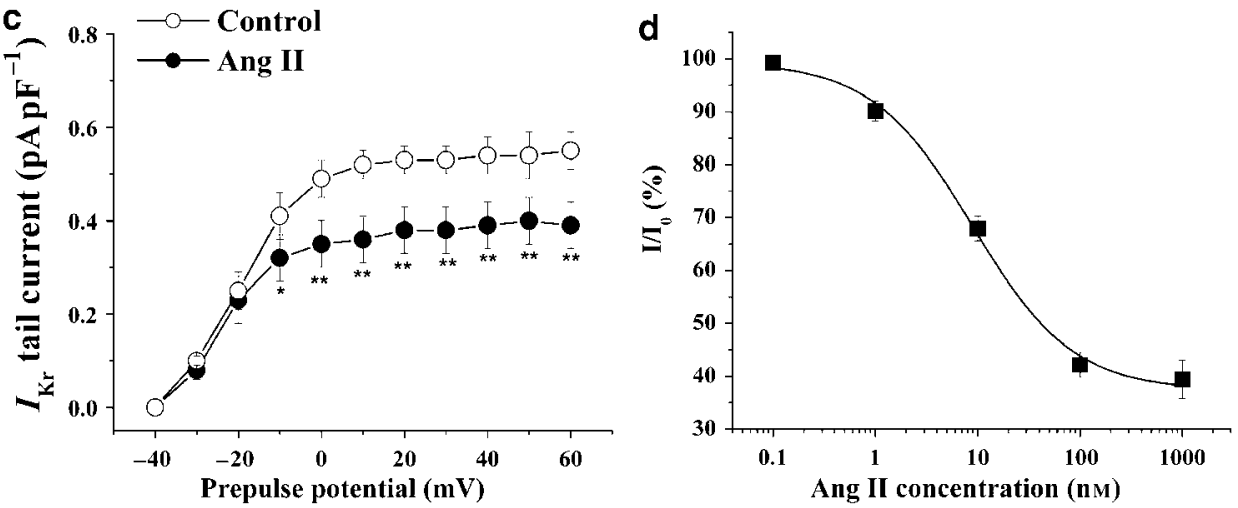


Figure 4. Voltage-dependent Activation of I_{Kr} in guinea-pig isolated ventricular cardiomyocytes. Left side figure: Voltage-dependent activation of I_{Kr} was calculated from I_{Kr} tail current density before and 10 min after an application of 100 nM Ang II (n=6, *P<0.05, **P<0.01, versus before Ang II). Right side figure: The concentration–response curve for the effect of Ang II on the I_{Kr}, IC50=8.9 nM (n=4–6 cells at each concentration).

Effects on Action Potentials: Ang II (100 nM) markedly prolonged APD, but did not influence the amplitude of the action potential and the resting potentials. In 10 myocytes, APD50 and APD90 were increased from the control values of 149.7 ± 10.4 ms and 184.2 ± 15.3 ms to 180.3 ± 12.3 ($p < 0.01$) and 210.8 ± 17.2 ms ($p < 0.01$), respectively. Furthermore, nimodipine, an L-type Ca^{2+} channel blocker, did not antagonize the prolongation of action potential by angiotensin II, suggesting that L-type Ca^{2+} currents are not involved in this effect of Ang II. It was abolished by losartan (1 mM), indicating that the effects were mediated through AT-1 receptors.

Effects of hERG Channels: Ang II decreased hERG current in HEK293 cells and significantly delayed channel activation, deactivation and recovery from inactivation. No effect of Ang II on IKr (hERG) currents was found in HEK293 cells unless AT1 receptors were co-expressed.

4.2.2 Effect of LJPC-501 on cloned hERG potassium channels expressed in human embryonic kidney cells

This GLP study (Study #160328.MBK; Sponsor document #TP 0100) was conducted at (b) (4), between (b) (4) The objective of the study was to evaluate the *in vitro* effects of LJPC-501 (Ang II) on human ether-a-go-go-related gene-encoded channels, which are the pore forming subunits of IKr. The I_{Kr} current is critical for repolarization of the cardiac action potential. Any blockade *in vivo* may cause prolongation of the QT interval.

Methods

For the assay, hERG potassium channels were expressed in a human embryonic kidney (HEK293) cell line. A primary stock solution of LJPC-501 (lot #2451-101, purity 101%) prepared in sterile water (2.53 mg/ml) and 25 mg/ml mannitol was delivered by the sponsor. Dilutions were made fresh daily in sterile water (3%v/v). HEPES-buffered physiological saline solution was used as the vehicle control for test article. Positive control (terfenadine) and reference substance (E-4031) were prepared in DMSO and aliquoted for individual use at the beginning of study. HEPES-buffered saline (pH 7.4) supplemented with 0.3% DMSO was used as the vehicle control for positive control.

The stability of the test article formulations was confirmed during the method validation study. For homogeneity determination, samples were collected from the top, middle and bottom of the formulation reservoirs. For concentration analyses, two samples were aliquoted on each day of testing from the outflow of the perfusion apparatus.

HEK-293 cells were stably transfected with hERG cDNA. Cells were mounted in a recording chamber and superfused with HEPES buffer at pH 7.4 (33 to 35ºC). The hERG tail current was recorded using the whole-cell patch clamp technique. Onset and steady state inhibition of peak tail current was measured in a vehicle control (n=4) and LJPC-501 solutions (at concentrations of 10 and 60 µM) (n=3). The performance of the system was tested with the application of the positive control (n=2). Duration of application was 4 min. Each recording ended with a final application of a supramaximal concentration of the reference substance (E-4031, 500 nM) to assess the contribution of endogenous currents. The remaining uninhibited current was subtracted off-line digitally from the data to determine the potency of the test substance for hERG inhibition.

Results

Based on the stability data, LJPC-501was stable over the conditions and duration of use in the present study. The concentration of LJPC-501 samples collected from the outflow were within ±15.0% of nominal. The solutions were homogenous and met the acceptance criteria (<15%).

LJPC-501 application reduced the amplitude of the outward tail currents by 1% at 60 µM. At the low concentration (10 µM), LJPC-501 inhibited hERG current by 1.8% (Table

5) (Fig. 5). Although IC_{50} was not calculated, on extrapolation it is greater than 60 µM, the highest tested concentration. The positive control, terfenadine (at 60 nM), produced 78.6 ± 7.3% mean inhibition of hERG potassium current, confirming the sensitivity of the test system to hERG inhibition.

Table 5. Mean percent inhibition at each LJPC-501 concentration

Concentration (µM)	Mean	SD	SEM	N
0	2.1%	1.6%	0.8%	4
10	1.8%	0.3%	0.2%	3
60	1.0%	4.5%	2.6%	3

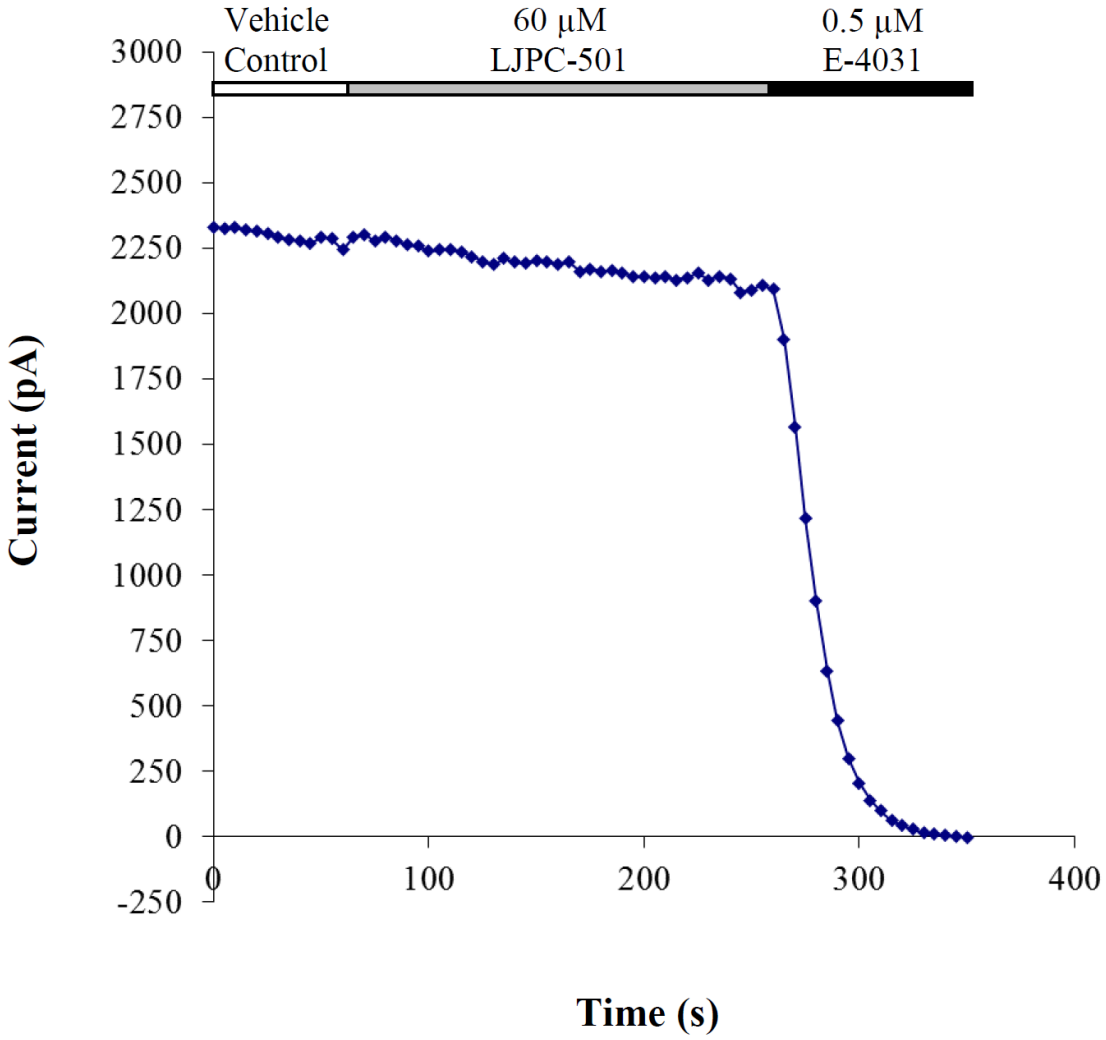


Figure 5. Typical time Course of the effect of LJPC-501 on hERG current.

Peak current amplitude during application of vehicle control, test article and reference substance is illustrated. The horizontal bars indicate the vehicle control, test article concentration and E-4031.

4.2.3 Cardiovascular effect of LJPC-501 on anesthetized beagle dogs

This GLP study (Study #355-1601; Sponsor document #TP 0095) was conducted at (b) (4), between (b) (4). The objective of the study was to evaluate the *in vivo* effects of LJPC-501 (Ang II) on cardiac contractility, hemodynamic, respiratory and electrocardiographic parameters in the anesthetized adult male beagle dog.

Methods: For the study, 12 male beagle dogs (12-14 months old, 7-15 kg) were obtained from (b) (4). Dogs were double-housed in pens with food and water available ad libitum. A primary stock solution of LJPC-501 (lot #2451-101, purity 101%) prepared in sterile water (2.53 mg/ml) and 25 mg/ml mannitol was delivered by the sponsor. Dilutions were made fresh daily in sterile saline to the final dose concentrations of 50, 150 and 600 ng/ml. On each day of dosing, 2 samples were collected from each dosing solution for concentration analysis.

Dogs received morphine (1 mg/kg, SC) 10-20 min prior to anesthetization with α-chloralose (120 mg/kg, IV). This was followed by a constant infusion of α- chloralose (35-75 mg/kg/hr, IV) administered through a cephalic or saphenous vein. Dogs were intubated and placed on a ventilator supplemented with oxygen to maintain arterial blood gases within the normal physiologic range and temperature was maintained at 37 ± 2°C. Under anesthesia, dogs were surgically instrumented for hemodynamic monitoring by isolating jugular vein and carotid artery. Surface ECG was continuously recorded via electrodes placed on the legs and chest. Test compound or saline were administered into the other side cephalic vein. Serum samples were collected from the cannulated femoral vein.

The study consisted of 2 treatment groups, vehicle and LJPC-501, with 6 animals in each group. After 30 min of equilibration period, a baseline serum sample was collected for determination of LJPC-501 followed by dosing of 4 increasing cumulative concentrations of LJPC-501 (0, 50, 150, 600 ng/ml for 30 min) or vehicle every 30 min (see Table 6 for the dose and the rate of infusion). At the conclusion of the final 30 min dosing period, a 30 min recovery period was initiated. The doses (150, 450 and 1800 ng/kg) and the rate of infusion were selected based on the information available from the published work.

Serum samples for determination of LJPC-501 were obtained at 5, 15 and 29 minutes after the initiation of dosing at each dose concentration and at 5, 15 and 29 minutes during the 30-minute recovery. Left ventricular pressure and systemic pressure were continuously recorded throughout the experiment. Hemodynamic parameters were recorded prior to the first dose and then at 5, 15 and 29 minutes of each dose level and at 5, 15 and 29 minutes of the 30 minute recovery period. Respiration rate (anesthetized) was recorded prior to the first dose and then at 5, 15 and 29 minutes of each dose level and at 5, 15 and 29 minutes of the 30 minute recovery period. At the end of the experiment (2.5 hr after the initiation of the first dose), dogs were euthanized and carcasses were disposed of without necropsy.

Table 6. Study design

Test Compound	Dose (ng/kg)	Dose Rate (ng/kg/min)	Dose Conc. (ng/mL)	Dose Volume (mL/kg/min)	Infusion Duration (min)	Number of Male Dogs
Vehicle[1]	0	0	0	0.1	30	6
	0	0	0	0.1	30	
	0	0	0	0.1	30	
	0	0	0	0.1	30[2]	
LJPC-501	0	0	0	0.1	30	6
	150	5	50	0.1	30	
	450	15	150	0.1	30	
	1800	60	600	0.1	30[2]	

[1]Vehicle is 0.9% NaCl, U.S.P. for injection.
[2] A 30 minute recovery period will initiate at the end of dosing.

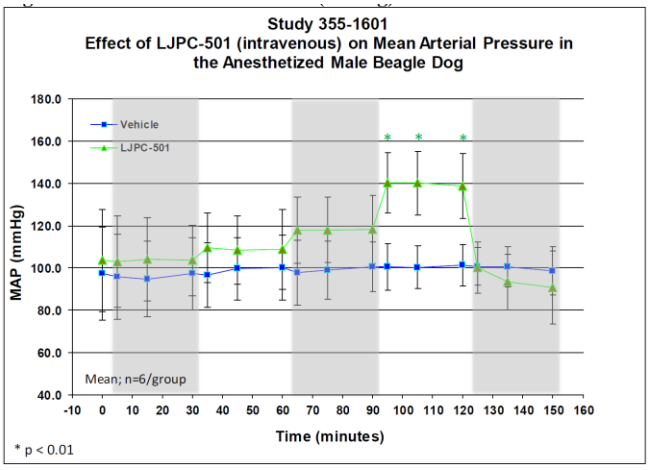
Results: The overall mean concentration of LJPC-501 of all formulations was within 16% of target concentration (Table 7).

Table 7. Concentration analysis of dose formulations

Angiotensin II Concentrations (ng/mL) in Dosing Solution (170045AQJG)						
Treatment	**Animal # 2887**	**Animal # 2893**	**Animal # 2883**	**Animal # 2884**	**Animal # 2886**	**Animal # 2880**
0 ng/mL	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ	BLOQ
50 ng/mL	56.84	56.71	50.59	68.81	46.19	61.1
150 ng/mL	178.25	182.58	154.5	201.73	137.04	198.44
600 ng/mL	687.32	767.52	626.11	846.27	578.11	730.27
BLOQ, below level of quanitifcation (<5.00 ng/mL)						

The systemic exposure to LJPC-501 to the dogs was measured by analyzing concentrations of LJPC-501 in serum samples taken at different intervals after the initiation of dosing. Toxicokinetic interpretation for this study was compromised because Ang II is not stable in whole blood or serum at room temperature or 4°C. The loss was estimated to be close to 90% before the samples were stabilized at the analysis laboratory. The only exposure data obtained at the highest infusion dose (1800 ng/kg) was highly variable with an AUC_{0-t} of 14,500 ± 3,330 min*pg/ml and Cmax of 473 ± 274 pg/ml (mean ± SD, n=2). Thus, the exposure at the lower doses was demonstrated by pharmacodynamic responses in pressor effects on mean arterial pressure at all doses (see Fig. 6).

The animals in the vehicle treated group had minimal cardiovascular changes in blood pressure, heart rate, systemic vascular resistance, cardiac function (cardiac output, LVSP, LVEDP, dP/dtmax, and dP/dtmin), electrocardiographic activity (PR interval, QRS duration, and QT/QTc interval), or respiratory rate from baseline during the 150 min test period. Treatment with LJPC-501 elicited dose-dependent cardiovascular changes from baseline over the 150 min dose period. LJPC-501 induced significant ($p<0.05$) dose-dependent elevation in mean arterial pressure (Fig. 6 left panel) and systemic vascular resistance (Fig. 6 right panel) at ≥ 450 ng/kg as well as an increase in heart rate at the highest dose. There were minimal to no significant changes in cardiac output, ventricular contractility (dP/dtmax) or ventricular relaxation (dP/dtmin). At the highest dose (1800 ng/kg), LJPC-501 induced statistically significant ($p<0.05$) and physiologically relevant increases in systemic vascular resistance, left ventricular systolic pressure (Fig. 7 left panel) and left ventricular end diastolic pressure (Fig. 7 right panel) with the greatest pressure increases of 29.7±4.3%, 33.0±4.6% and ~125±12%, respectively. There were no statistically significant ECG changes in QRS duration or QTcV interval. However, PR interval prolongation (14 ± 1.5%; 14 ± 5.8 msec; $p<0.05$) (Fig. 8 right panel) was noted during and following the maximum dose in accordance with increases in HR (Fig. 8 left panel). LJPC-501 did not alter respiratory rate in freely breathing intubated, anesthetized animals. The lowest dose (150 ng/kg) did not elicit any statistically significant or physiologically relevant hemodynamic, cardiac function, ECG or respiratory effects.



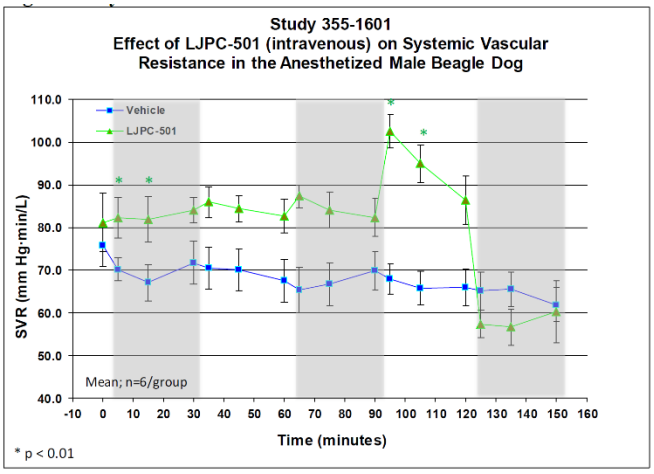
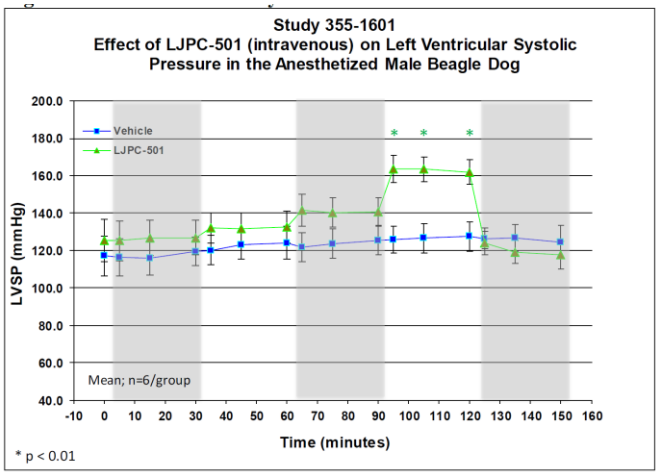


Figure 6. Effect of LJPC-501 on mean arterial pressure (left panel) and systemic vascular resistance (right) in the anesthetized dog.

As noted in methods, dogs were dosed LJPC-501 in 4 increasing cumulative concentrations of 0, 50, 150 and 600 ng/ml for 30 min followed by a 30 min recovery period. Each of these are represented by a vertical box (30 min duration) in the figure starting at 0 min.



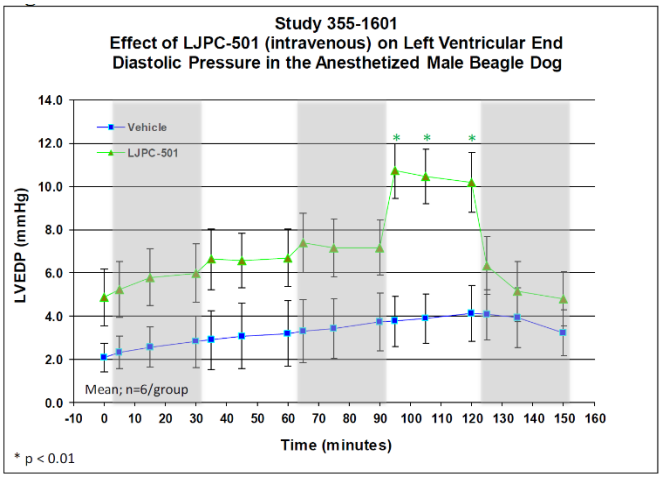
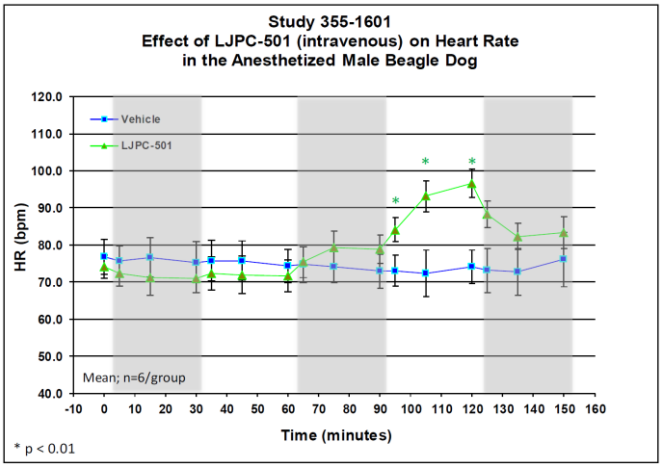


Figure 7. Effect of LJPC-501 on left ventricular systolic pressure (left panel) and left ventricular end diastolic pressure (right) in the anesthetized dog



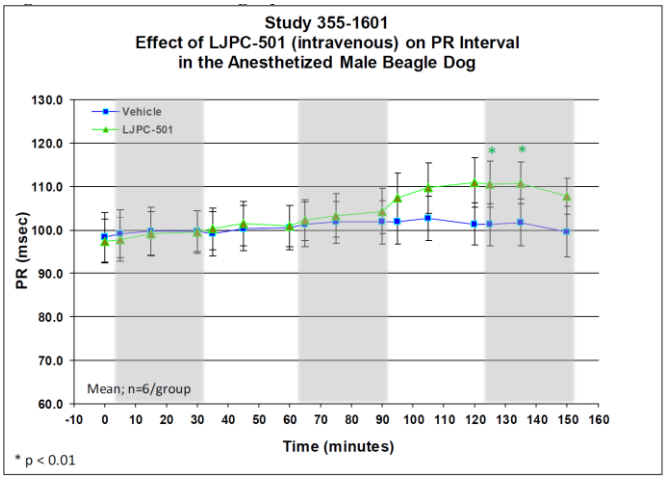


Figure 8. Effect of LJPC-501 on heart rate (left panel) and PR interval (right) in the anesthetized dog

4.2.4 Prothrombotic effect of Angiotensin II

The sponsor has not addressed in the submission on the prothrombotic effects of renin angiotensin aldosterone system (RAAS) in general, and its key mediator Ang II, in specific. A literature search by this reviewer shows the prothrombotic effect of Ang II and aldosterone in animals and humans. Key evidences are summarized from publications (listed below) and are referenced by numbers in the text.

Hypertension is commonly associated with abnormalities in platelet function (reactivity/activation), coagulation and fibrinolysis that could lead to increased risk of thrombus formation (1,2) as there are increased circulating levels of tissue factor (TF) and thrombogenic microparticles (3) in hypertensive patients. This feature is considered as a risk factor for myocardial infarction or stroke (3-5). The prothrombotic effect of Ang II, Ang (1-9) and aldosterone has been demonstrated in animals (2,7-9), and Ang II in humans (11,14,15,18). RAS inhibitors (ACEi, ARBs and renin inhibitor) and eplerenone (mineralocorticoid receptor antagonist) have demonstrated antithrombotic and fibrinolysis activities; thus, reducing the risk of ischemic complications in hypertensive subjects (3,10-13).

The mechanism by which Ang II contributes to a prothrombotic state was investigated in healthy volunteers. A dose-dependent increase in plasminogen activator inhibitor-1 (PAI-1, the most important physiological inhibitor of plasminogen activator (t-PA) that activates fibrinolytic system) was noted in normotensive subjects receiving 1, 3, or 10 ng/kg/min Ang II infusion for 45 min relative to control subjects. There was no effect on t-PA in plasma (14). However, short term infusion of 10 ng/kg/min for 15 min did not have a statistically significant effect relative to control subjects on PAI-1 or t-PA (15). In hypertensive subjects, increased circulating levels of tissue factor (TF), the physiologic initiator of blood coagulation (11) is reported. Several clinical and non-clinical studies have connected Ang II to TF synthesis (reviewed in ref 11). The transcription factor, NF-κB, largely regulates TF gene promoter (16). Ang II upon binding to AT-1 receptors activates NF-κB and in turn promotes synthesis of TF. In hypertensive patients, treatment with three different ARBs (losartan, irbesartan and candesartan) for 2 months reduced blood pressure and additionally, reduced TF activity and PAI-1 antigen (13). Another risk factor for increased risk for thrombosis is alterations in platelet function. In vitro studies show that Ang II at picomolar levels induces rapid aggregation of murine platelets with AT-1 receptor activation on platelets (1). Infusion of Ang II in animals (1) and healthy volunteers (10 ng/kg/min for 15 min) (17) resulted in platelet activation and increased thrombin formation.

In summary, Ang II stimulates the production of PAI-1, sensitizes platelets, promotes the production of superoxide radicals that scavenge free NO, and activates NF-κB that increases the expression of TF. Collectively, these processes would have impact on the balance between clotting and fibrinolytic mechanisms for thrombotic tendency.

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5 Pharmacokinetics/ADME/Toxicokinetics

No stand-alone PK studies were conducted by the sponsor on LJPC-501. The Agency agreed that the information in the literature is sufficient and no nonclinical PK studies with LJPC-501 are required. A short summary based on the published literature is given in section 10, Integrated Summary and Safety Evaluation.

6 General Toxicology

No stand-alone toxicology studies were conducted by the sponsor on LJPC-501. Results and the follow up discussion for each study are based on the published articles included in the submission.

6.1 Effects of single doses of Val5-angiotensin II-amide on the kidneys of normal and hypertensive rats

Reference: Byrom FB. Angiotensin and renal vascular damage. *Br. J Exp Pathol,* **45**: 7-12 (1964).

Purpose: To study the effects of single intravenous doses of Val5-angiotensin II-amide from 10 min to 7 hr on the kidneys of normal and hypertensive rats.

Methods: a) Normal rats: 40 young females weighing 180 gm; 14 males, large, 6-18 months old. The effects of very large doses (1-100 µg/rat) of Val5-angiotensin II-amide (Hypertensin®) were evaluated on the kidney morphology of normal rats. Ang II was administered as follows:

- A single dose (11 rats)
- Two doses separated by an interval of 10 minutes (21 rats)
- Several doses at 30 min intervals over periods from 2.5 to 7 hours (9 rats)
- A continuous infusion at a rate of 0.01 to 5.0 µg per minute with or without chloralose and pentobarbital anesthesia (13 rats)

In most experiments the left kidney was excised for histology immediately after the blood pressure returned to normal. The right kidney was excised on the day following the injection of Ang II.

b) Hypertensive rats: IV administration of Ang II, 10 to 20 µg in 2 or 3 doses, separated by intervals of 10 min to one of the two hypertensive model rats.

- Hypertension derived from a solitary kidney - Chronic renal hypertension was caused by removing the right kidney and constricting the left renal artery, and

allowing hypertension to persist for 5 to 40 weeks before the injection of Ang II (10 rats).

- Unilateral renal hypertension - Chronic renal hypertension was caused by a "clipped" left and an intact right kidney, and allowing hypertension to persist for 22 to 38 weeks before the injection of angiotensin II (5 rats).

Animals were sacrificed on the day following injection of Ang II.

Results: a) Normal rats: Detectable rises in blood pressure were noted as doses as little as 2 ng for 10 min. In all Ang II treatment groups, the right kidney (excised immediately after the blood pressure returned to normal) showed necrosis of the arteries in the hilum, primarily in the large but occasionally in the smaller branches. The necrosis was medial and involved part or the whole circumference of the vessel. Both aneurysms and focal necrosis were seen in these arteries. Necrosis was not seen in the renal cortical tubules. Apart from occasional glomerular aneurysms, the left kidney was free from the changes described for the right kidney, except in 3 rats that received multiple injections of Ang II over at least 3 hours. Although necrosis was absent, the hilar arteries were often dilated and thin-walled with stretched (but intact) internal elastic lamina. These effects were attributed to the physical stress caused by overstimulation and excessive filling tension from Ang II.

b) Hypertensive Rats: In 9 of the 10 hypertensive rats where hypertension was derived from a solitary kidney, arterial necrosis was found in the solitary kidney and in some cases involved not only the hilar arteries but also medium sized and even terminal vessels, and in three rats numerous glomerular aneurysms and necrosis were found. In rats with chronic unilateral renal hypertension, arterial necrosis was found in the large and medium sized renal arteries in the “clipped” left kidney. In the right kidney, the large arteries were all completely free from necrosis but showed a very impressive degree of medial hypertrophy.

6.2 Nitric oxide synthase inhibition accelerates the pressor response to Ang II

Reference: Hu L, et al. Nitric oxide synthase inhibition accelerates the pressor response to low-dose Angiotensin II, exacerbates target organ damage, and induces renin escape. *Am J Hyptn,* **17**:395–403 (2004).

Purpose: To study the effects of repeat intravenous doses of Ang II on normal rats. Additionally, the study investigated whether nitric oxide synthase (NOS) inhibition (i.e., reduced the availability of NO) accelerates the slow pressor effect of Ang II and augments organ damage in normotensive rats.

Methods: Sprague-Dawley rats were chronically implanted with arterial and venous catheters for blood pressure and monitoring and infusions. After 7 days of recovery, the animals were treated as follows.

- Control (n = 11): After an additional 5-day control period, saline was administered by IV infusion for 7 days followed by IV infusion of Ang II (10 ng/kg/min) for 14 days.
- L-NAME alone (n = 8): After an additional 5-day control period, N-nitro-L-arginine methyl ester (L-NAME [an inhibitor of NOS], 10 µg/kg/min) was administered by IV infusion for 7 days.
- L-NAME plus angiotensin II (n = 15): After an additional 5-day control period N-nitro- L-arginine methyl ester (L-NAME, 10 µg/kg/min) was administered by IV infusion for 7 days followed by IV infusion of Ang II (10 ng/kg/min) for 14 days.
- Ang II alone (n = 14): The angiotensin II infusion (10 ng/kg/min) was initiated immediately after the 7-day recovery period for 14 days.

Heart and kidneys were collected from each animal and histopathology analyses were performed. Blood samples were collected daily, at noon, to measure plasma renin concentration (PRC) cardiac troponin T (cTnT). Urinary protein was also measured.

Results: Ang II alone increased BP to in two separate phases. Mean arterial pressure increased 22 mm Hg on the first day, and another 6 mm Hg on the second day (study days 15 and 16, respectively). Mean arterial pressure remained at 28 mm Hg above baseline through the fourth day and then increased again during days 5 to 9 by another 25 mm Hg. Mean arterial pressure remained constant for the final 5 days of the Ang II infusion at about 53 mm Hg above baseline (Fig. 9). Ang II did not cause vascular, cardiac or kidney toxicity at this low dose.

L-NAME alone increased MAP by 16 to 20 mm Hg on first 2 days. Mean arterial pressure remained constant until days 14 to 16 (study days 21 to 23), and began to increase again. Addition of Ang II to L-NAME, MAP increased by 29 mmHg on day 1 and by 4 to 6 mmHg per day on days 2 to 4, reaching a plateau as high as that reached on Day 9 of Angiotensin II alone, suggesting additive effect of these two agents on MAP (Fig. 9).

Kidneys of rats treated with Ang II or L-NAME alone were histologically similar to control rats. However, kidneys of L-NAME plus Ang II-treated rats, showed extensive perivascular fibrosis, glomerular fibrosis, and tubulointerstitial damage. Similarly, hearts of animals treated with either L-NAME or Ang II alone were histologically similar to control rats, whereas hearts of L-NAME plus Ang II-treated rats, exhibited marked perivascular and interstitial fibrosis, and arterial wall thickening. On the first day that Ang II was added to L-NAME, urinary protein excretion and plasma cardiac troponin T increased, indicating early target organ damage. By the end of the study, all rats treated with L-NAME plus Ang II developed tubulointerstitial and glomerular injuries, fibrosis of the renal and cardiac arteries, and cardiac interstitial fibrosis. It was minimal in rats infused with Ang II or L-NAME alone.

There was no detectable cTnT in the plasma with saline, L-NAME or Ang II (10 ng/kg/min) infusion. However, simultaneous infusion of Ang II and L-NAME caused a rapid and consistent increase in plasma cTnT, which was detectable on day 1, peaked on days 1 and 2, and steadily declined thereafter, but never returned to baseline. Similarly, remarkable changes in protein excretion were observed in L-NAME plus Ang II-treated animals. Ang II alone had no effect on urinary protein excretion in the first week, but increased 2- to 3-fold relative to control during the 2nd week of Ang II treatment.

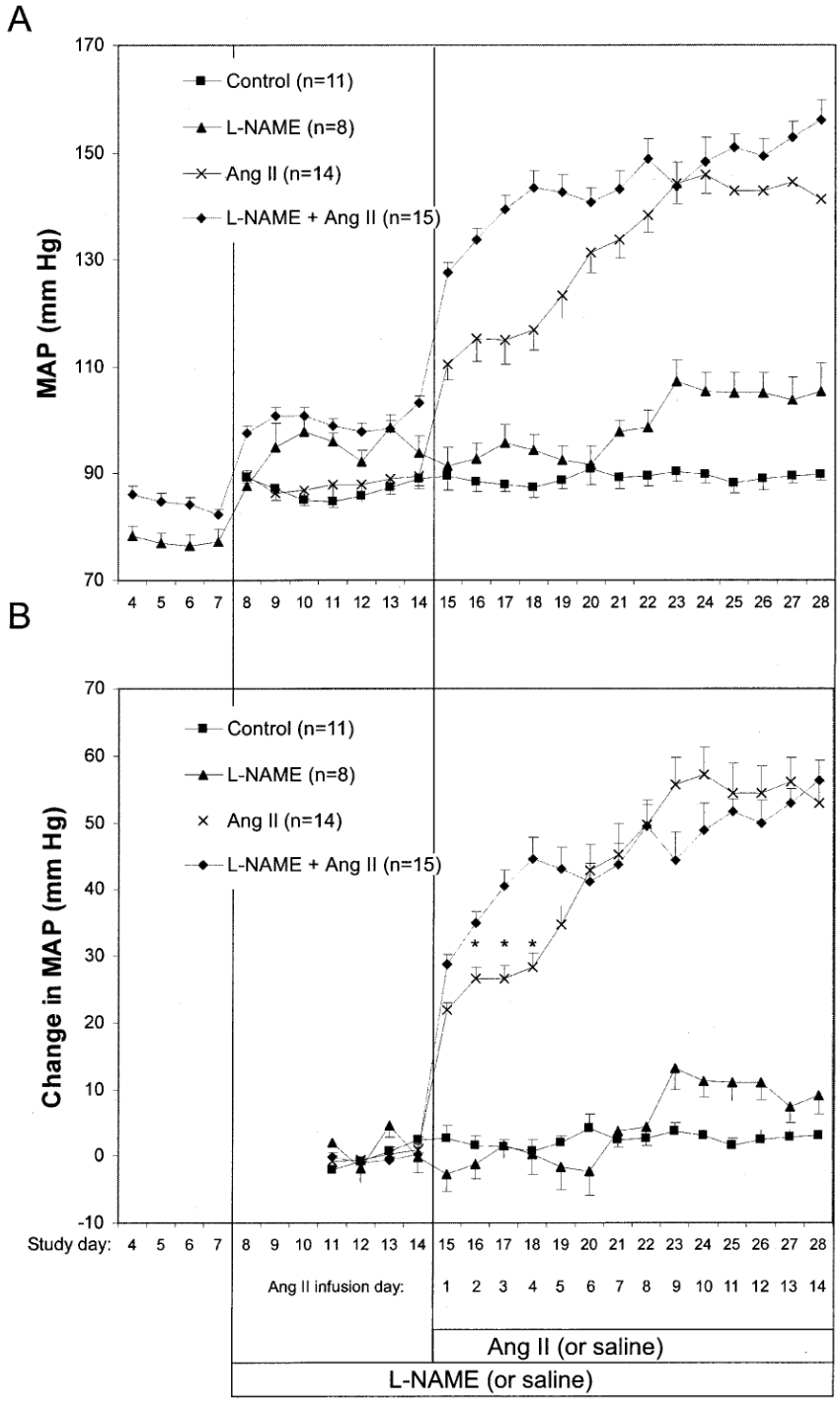

Figure 9. Effects of Ang II and N-nitro-L-arginine methyl ester (L-NAME) infusion, alone or in combination, on mean arterial blood pressure (MAP).

<u>Conclusions:</u>
Blockade of NO production during angiotensin II infusion causes blood pressure to increase more rapidly and results in rapid and extensive damage to the heart and kidneys, which does not occur when either L-NAME (NO blockade) or Ang II are administered alone over the same duration. These observations suggest that under conditions of reduced NO bioavailability even small increases in angiotensin II may result in target organ damage. This suggests that endogenous NO normally protects against

both the pressor and cytotoxic effects of small increases in plasma Ang II. A close interrelationship appears to exist between both the pressor and cytotoxic effects of Ang II and NO inhibition.

6.3 Effect of Ang II on the heart and kidneys of normal rabbits

Reference: Kremer D, et al. Angiotensin-induced myocardial necrosis and renal failure in the rabbit: distribution of lesions and severity in relation to plasma angiotensin II concentration and arterial pressure. *Cardiovasc Res,* **15**:43–6 (1981).

Purpose: To evaluate the relationship between myocardial and renal lesions, and arterial plasma Ang II levels.

Methods: New Zealand white rabbits weighing 1.5 to 4.5 kg were catheterized in the carotid artery for blood sampling or for direct blood pressure recording, and a jugular vein for continuous intravenous infusions of 0.9 % saline or Asn1-Val5-Ang II amide (Hypertensin® from Ciba). Infusion rates varied in different animals from 1 to 500 ng/kg/min and maintained for 24 hr. Arterial blood samples, for estimation of plasma angiotensin II, urea and electrolytes, were drawn from the carotid catheter, immediately before the end of the control period on saline alone, and again after 6 and 24 h of infusion of Ang II (or control infusion of saline). Rabbits were killed 24 h after completion of the infusion. The heart and kidneys were harvested for histopathological examination.

Results: The intravenous infusion of Ang II (1 to 500 ng/kg/min) caused dose-related increases in arterial plasma Ang II concentrations, from control values in the range 10 to 100 pmol/L to a maximum of 35,000 pmol/L at the highest rates of infusion (Fig. 10). Increases in arterial blood pressure to a maximum of 40 mm Hg were in proportion to the arterial concentrations of Ang II achieved. The logarithm of the mean arterial plasma Ang II concentration noted during the infusion was significantly related ($p < 0.001$, $r = +0.83$) to the change in mean arterial pressure.

Ang II-induced myocardial necroses was predominantly observed in the left ventricle. Effects on the right ventricle were noted at the highest Ang II doses. There were no effects in the endocardium, arteries and arterioles. Also, there was absence of fibrosis and vascular congestion. The necrotic areas were surrounded by a mononuclear cell reaction, mainly histiocytes and fibroblasts, some of which showed mitotic figures. Myocardial necroses were observed over a wide range of arterial plasma Ang II, but were absent below 200 pmol/L. The logarithm of the mean infusion plasma Ang II concentration was significantly correlated ($r = +0.84$; $P < 0.001$) to cardiac histopathology toxicity. In addition, blood pressure and cardiac toxicity was significantly correlated ($r = +0.92$; $P < 0.001$).

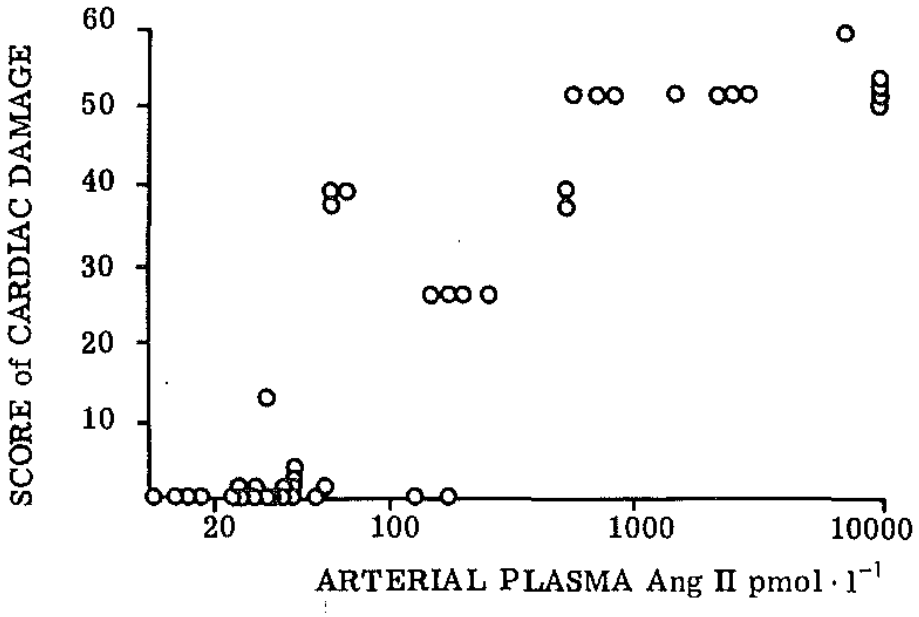

Figure 10. Severity of myocardial lesions (assessed as an arbitrary points "score") in relation to mean infusion arterial plasma Ang II concentration.

Ischemic necroses were observed in the proximal, distal and collecting tubules of kidneys of rabbits receiving Ang II. In these animals, plasma urea concentration increased from control values, ranging from 3.0 to 10.0 mmol/L to 11.6 to 15.0 mmol/L at 6 hours and 23.25 to 33.25 mmol/L at 24 hours. The logarithm of the mean infusion arterial plasma Ang II concentration was significantly correlated ($p < 0.001$, $r = 0.85$) to the plasma urea level at the 24th hour of infusion.

In summary, IV administration of large doses of Ang II in the rabbit induces multifocal myocardial necrosis, together with renal failure and renal tubular necrosis. The renal and cardiac abnormalities were correlated with the pressor effects of administered Ang II. The severity of the renal and cardiac lesions, the elevation of arterial plasma Ang II and blood urea, and the increment of arterial pressure, showed significant correlation.

6.4 Effect of Ang II on the heart and kidneys of normal rabbits

Reference: Gavras LA, et al. Acute renal failure, tubular necrosis, and myocardial infarction induced in the rabbit by intravenous angiotensin II. *Lancet* **298** (7714):19–22 (1971).

Purpose: To evaluate the myocardial and renal effects of large doses of intravenous Ang II in conscious rabbits.

Methods: New Zealand white rabbits weighing 2.5 to 4.0 kg were catheterized in the jugular vein for continuous infusion of Ang II (Asn1-Val5-angiotensin II-amide

(Hypertensin®, Ciba Geigy)) (0.9-1.8 µg/kg/min for 72 hours). Continuous 24-hour urine was collected with the measurement of sodium and potassium content. With the three experimental procedures, 15 rabbits were studied.

Group 1: Ang II infusion with no sodium supplement: Initially, five rabbits were infused with 0.9% saline solution for 72 hours followed by blood sampling from an ear artery for serum urea, creatinine, and electrolyte estimations. The animals were then infused with Ang II, 0.9-1.8 µg/kg/min, for 72 hours. Blood samples were taken at 24, 48, and 72 hours from the start of the Ang II infusion. At the end of infusion, rabbits were killed, and heart and kidneys were removed for histopathological examination.

Group 2: Ang II infusion with sodium supplement: Five rabbits were treated as described for Group 1, except they were given a subcutaneous injection of hypertonic saline solution (50 mEq sodium per 100 ml; 10-50 mEq) immediately before the start of the Ang II infusion, and again each day until the end of the experiment to keep the animals in positive sodium balance throughout the study. Blood samples were taken at 48 and 72 hours from the start of the Ang II infusion.

Group 3: Sodium deficit and no infusions: Five rabbits were maintained for 5 days on a diet of normal sodium and potassium content, after which blood-samples were collected for urea, creatinine, and electrolyte determination. Following this, animals received a sodium-deficient diet (< 1.0 meq. daily) for 3 days, and a daily intramuscular injection of 20 mg furosemide to produce a mean sodium deficit slightly in excess of that in the animals of Group 1. At the end of 3 days, blood samples were collected; the animals were killed, and the kidneys and hearts were removed for histopathological examination.

Results: Ang II infusion increased blood pressure in animals receiving sodium supplementation (group 2) or not (group 1). Blood pressure returned to basal levels at the end of the experiment. Additionally, these animals ate less food with the start of Ang II infusion. The effect Ang II on kidney function was similar in both groups with an increase in serum urea by approximately 4-fold as was a potassium deficit. The ratios of urine/serum urea and urine/serum creatinine also decreased for both groups (Table 8). Histologically, kidney tubular necrosis was observed in rabbits from both groups, irrespective of sodium supplementation. These effects were noted in both the proximal and distal tubules with the glomeruli and arterioles being unaffected. Additionally, most of the rabbits in groups 1 and 2 had widespread focal myocardial infarction although no arterial lesions were seen. No changes in serum urea, renal and cardiac lesions were observed in control furosemide treated group 3 animals.

In summary, the study demonstrated acute renal failure with uremia, tubular necrosis, and myocardial infarction after IV infusion of Ang II in the dose range of 0.9 to 1.8 µg/kg/min for 3 days.

Table 8. Effects of repeat-dosing of intravenous Ang II in rabbits

Group	Serum-urea (mg/100 mL)		Urine/serum-urea ratio		Urine/serum-creatinine ratio		24-hour urine volume (mL)		Serum-sodium (meq/L)	
	Control Period	Exp Period	Control Period	Exp Period	Control Period	Exp Period	Control Period	Exp Period	Control Period	Exp Period
Ang II infusion w/o sodium supplement	40.60 ± 1.69	198.00 ± 30.81[a]	0.42 ± 0.12	0.05 ± 0.01[b]	63.00 ± 13.01	14.20 ± 4.93[c]	164.1 ± 37.1	188.8 ± 25.8	139.60 ± 2.78	132.50 ± 1.84
Ang II infusion with sodium supplement	43.40 ± 8.20	172.00 ± 33.74[a]	0.44 ± 0.16	0.07 ± 0.01[b]	89.25 ± 34.02	28.37 ± 10.27	192.3 ± 39.1	254.3 ± 51.3	139.61 ± 2.99	142.25 ± 3.42
Low sodium diet with daily frusemide	46.00 ± 7.37	55.40 ± 7.54	0.49 ± 0.15	0.21 ± 0.05	36.26 ± 13.94	46.87 ± 13.84	239.7 ± 23.0	261.5 ± 38.3	146.20 ± 3.58	133.66 ±1.76[b]

Group	Serum-potassium (meq/L)		Serum-bicarbonate (meq/L)		Cumulative sodium balance (meq)		Cumulative potassium balance (meq)		Rabbits with tubular necrosis	Rabbits with MI
	Control Period	Exp Period	Control Period	Exp Period	Control Period	Exp Period	Control Period	Exp Period	Control Period	Exp Period
Ang II infusion w/o sodium supplement	4.86 ± 0.26	3.42 ± 0.14[a]	17.2 ± 1.57	15.25 ± 1.75	-13.97 ± 4.63	-24.41 ±6.96	-5.07 ± 5.15	-21.25 ± 3.06[b]	3/5	3/3
Ang II infusion with sodium supplement	4.86 ± 0.57	4.20 ± 0.86	20.00 ± 1.62	14.75 ± 1.65[b]	-5.92 ± 3.59	29.96 ± 8.24[a]	3.12 ± 3.52	-14.99 ± 4.48[c]	5/5	3/4
Low sodium diet with daily frusemide	4.60 ± 0.20	3.73 ± 0.73	17.70 ± 1.63	16.83 ± 3.11	-3.84 ± 5.12	-37.21 ± 4.36[d]	26.56 ± 7.27	-21.73 ± 3.62[d]	0/5	0/5

Ang II = Angiotensin II; Exp = experimental; MI = myocardial infarction
Serum values and ratios shown are those on the final day of the respective period; urine volumes and cumulative balances are means for the whole of that period. Differences between means in the Control Period and Experimental Period were significant at the following p-levels (t test):
A: $p < 0.01$; b: $p < 0.05$; c: $p < 0.02$; d: $p < 0.001$

6.5 Ang II induces acute coronary syndrome in hyperlipidemic rabbits

Reference: Li S, et al. Angiotensin II destabilizes coronary plaques in Watanabe heritable hyperlipidemic rabbits. *Arterioscler Thromb Vasc Biol,* **36**:810-816 (2016).

Purpose: To evaluate whether elevated plasma Ang II can directly induce coronary events, such as acute coronary syndrome.

Methods: Watanabe heritable hyperlipidemic (WHHL) rabbits (8 months old), a model of human familial hypercholesterolemia, which spontaneously develop coronary atherosclerosis, were used. Under anesthesia, mini osmotic pups were placed in the subcutaneous space through a small incision on the back of the neck. The pumps contained either saline or different doses of Ang II (Fig. 11). Osmotic pumps were changed at 4 weeks. Two types of experiments were conducted based on the method of administration (rapid or slow infusion) of Ile5-Ang II.

Experiment 1: Examine the effects of rapid elevation of plasma Ang II

WHHL rabbits were infused with either of two doses of Ang II or saline for 4 weeks (Fig. 11 Top panel):

- 100 ng/kg/min angiotensin II (N = 10, designated as low-Ang II)
- 200 ng/min/kg angiotensin II (N = 12, designated as high-Ang II)
- Saline vehicle (N = 8)

Experiment 2: Examine the effects of gradual elevation of plasma Angiotensin II

WHHL rabbits were first infused with 50 ng/min/kg Ang II (N = 7, low-Ang II) or 75 ng/min/kg Ang II (N = 7, high-Ang II) for 4 weeks and then further infused with 100 ng/min/kg Ang II or 150 ng/min/kg Ang II, respectively, for another 4 weeks. The vehicle group (N = 6) was continuously infused with saline for 8 weeks with osmotic pumps changed at 4 weeks (Fig. 11 Bottom panel).

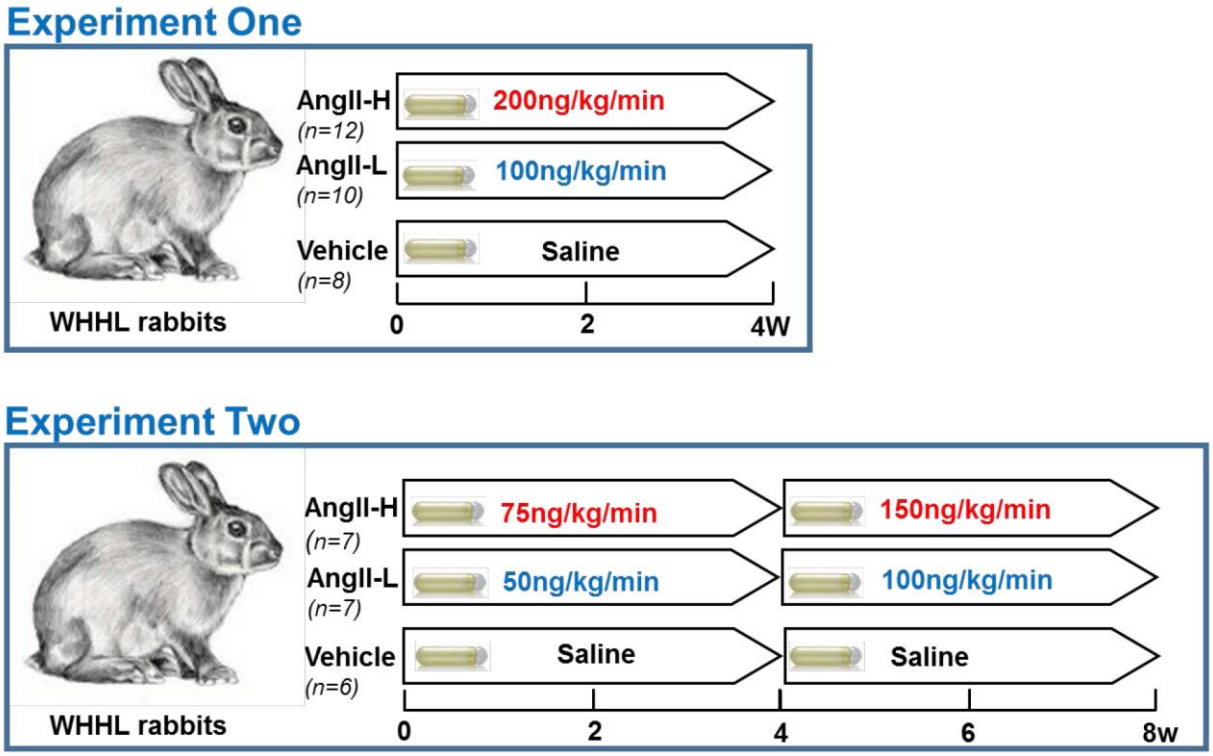

Figure 11. Experimental design.

Two experiments were designed to illustrate the effects of Ang II infusion administered differently: acute increase of Ang II in circulation by a single high-dose infusion (upper panel) and escalation increase of Ang II in circulation by two different doses (from low- to high-dose infusion) (lower panel).

The medial auricular artery was cannulated and BP was simultaneously recorded. The data were collected for 15 to 25 minutes after rabbits became completely

calm using a BP amplifier. Blood was collected from rabbits after 16 hours of food deprivation. Plasma lipids and hematological examinations were performed.

Pathological Examinations: Rabbits that died during the study were necropsied 3 to 10 hours after death. It included gross and microscopic examinations of thoracic and abdominal cavity, and all important organs (liver, adrenal, spleen, kidneys, stomach and intestines, heart and lung, brain). Additionally, these organs were fixed for microscopic examination. Rabbits that survived to the end of the experiment were euthanized and tissues from lung, heart and aorta, brain, liver, and kidney were collected for histopathologic examination.

The aortic trees were isolated, opened out, and fixed. For the microscopic quantification of the lesion areas and lesion features, aorta was divided into arch, thoracic and abdominal segments, and each segment was further cut into cross sections. All sections were stained for immunohistochemistry. In addition, a small piece of the aortic arch was collected for extraction of total RNA and the mRNA expression of cytokines (plasminogen activator inhibitor-1 [PAI-1], interleukin-1β [IL-1β], IL-6, tumor necrosis factor-α [TNF-α], monocyte chemoattractant protein-1 [MCP-1], matrix metalloproteinase-9 [MMP-9], collagen I and collagen III).

For the analysis of coronary lesions, hearts were dissected into 5 blocks. Blocks I and II contain the main trunks of the left and right coronary arteries. To undertake an extensive examination of coronary lesions, these two blocks were cut into 4 serial sections. Blocks III through V were cut into 3 serial sections. These sections were stained and their histological features (presence of myocardial infarction and atherosclerosis) were examined under light microscopy. Coronary lesions of blocks I through V were quantified for the following:

- The number of plaque erosions (i.e., those lesions where the intimal surface was eroded and the maximal depth was > 15 μm) and ruptures (i.e., those lesions that show apparent split of disruption of the fibrous cap accompanied by thrombosis or the intrusion of blood cells), with and without thrombosis in each block
- Lumen stenosis in Blocks I and II.

Serial sections adjacent to sections with erosion or rupture features were selected and immunohistochemically stained with antibodies against rabbit macrophage (RAM-11), α-smooth muscle actin (HHF-35), MMP-1, MMP-2, MMP-9, and MMP-12.

Results: The most prominent feature after Ang II administration was gradual death of WHHL rabbits in both experiments. In Expt. 1, the mortality rates reached 50% (5/10) in the low-Ang II group at 4 weeks and 92% (11/12) in the high-Ang II group by 16 days (Fig. 12). These rabbits died at various times, whereas none of the vehicle rabbits died. All dead rabbits in both low-Ang II and high-Ang II groups showed severe pulmonary edema, congestion, and hemorrhage, which were not present in all surviving rabbits euthanized at 4 weeks. The pulmonary pathological features suggested that the death of the WHHL rabbits was possibly caused by acute left heart failure. There were no

abnormalities in other organs, such as liver, kidneys, brain, adrenals, stomach, and intestines.

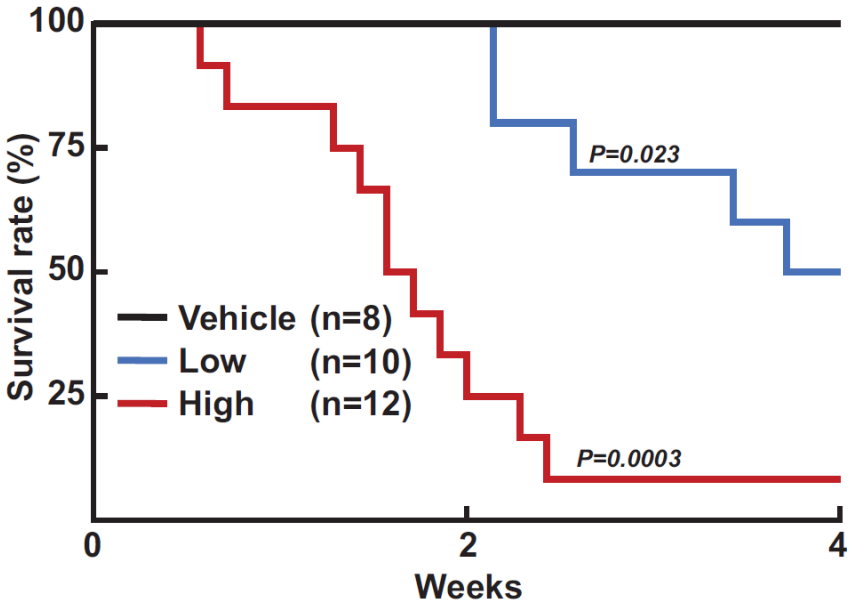

Figure 12. Experiment 1. Ang II infusion induces a high mortality rate.

Kaplan–Meier analysis of cumulative rates of survival in WHHL rabbits after Ang II rapid infusion.

Many rabbits looked inactive, showed dyspnea, and ate less food before they died, but 2 healthy rabbits died suddenly. Surviving rabbits of the Ang II groups at 4 weeks showed high blood pressure along with an increased number of blood neutrophils and monocytes relative to the vehicle group (Fig. 13).

Since Ang II infusion led to a high prevalence of MI and a high mortality rate, the authors examined whether coronary plaque erosion/rupture was present, which may be responsible for MI observed in Ang II–infused WHHL rabbits. Although coronary atherosclerosis was observed in different-sized arteries varying from large epicardial arteries (blocks I and II) to small arteries and arterioles (blocks III–V), the lesions of epicardial arteries in blocks I (left coronary artery) and II (right coronary artery) were consistently present in all rabbits (Table 9). These lesions were characterized either by fibrosis with more smooth muscle cells and few macrophages or by the accumulation of foam cells on the lumen surface or a typical necrotic core covered by a thin fibrous cap. Coronary plaque erosion/rupture along with thrombosis were present in both low- and high-Ang II groups but not in the vehicle group. Additionally, matrix metalloproteinases (MMP) were involved in the plaque rupture and thrombosis. In summary, Experiment 1 described above showed that the abrupt increase of plasma Ang II resulted in high prevalence of MI and death, which may possibly be caused by or related with coronary atherosclerosis.

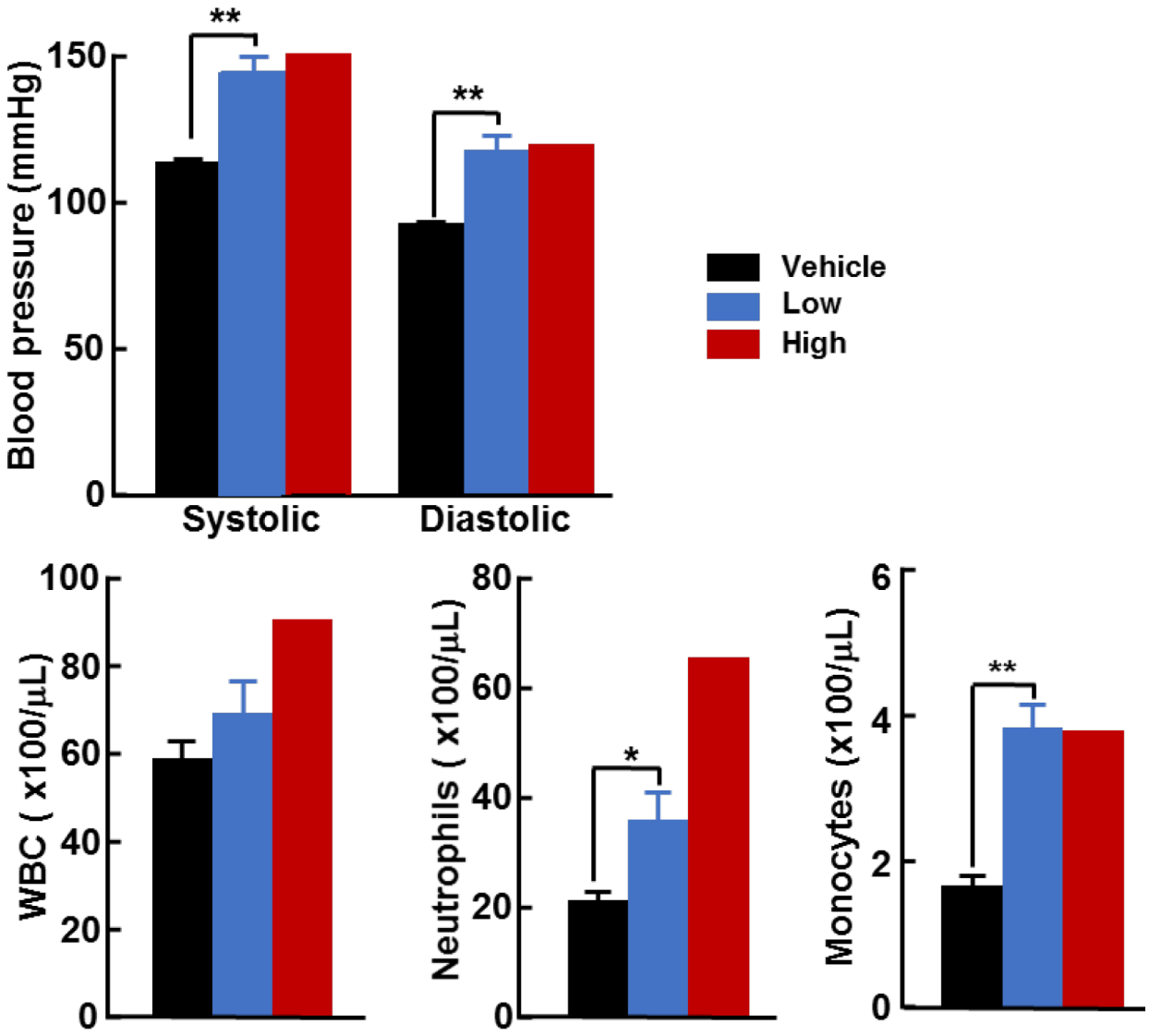

Figure 13. Experiment 1. Blood pressure (upper) and blood leukocyte count (lower panel) measured at 4 weeks after Ang II infusion.

N=5 for low-Ang II and N=1 for high-Ang II groups. Data are expressed as mean ± SEM. * P<0.05 or ** P<0.01 versus the vehicle (n=7).

Table 9. Experiment 1. Number of coronary erosion, rupture, and thrombosis in each block

	Block I	Block II	Block III	Block IV	Block V
Coronary Erosion					
Vehicle (n=8)	0±0 (0/8)	0±0 (0/8)	0±0 (0/8)	0±0 (0/8)	0±0 (0/8)
Ang II-L (n=10)	1±1.25 (5/10)	0.7±0.68* (6/10)	0.4±0.97 (2/10)	0±0 (0/10)	0±0 (0/10)
Ang II-H (n=12)	1.08±0.9** (9/12)	1.0±1.04* (7/12)	0.73±0.65** (7/12)	0±0 (0/12)	0±0 (0/12)
Ruptures					
Vehicle (n=8)	0±0 (0/8)	0±0 (0/8)	0±0 (0/8)	0±0 (0/8)	0±0 (0/8)
Ang II-L (n=10)	0±0 (0/10)	0±0 (0/10)	0.1±0.31 (1/10)	0±0 (0/10)	0±0 (0/10)
Ang II-H (n=12)	0.17±0.58 (1/12)	0±0 (0/12)	0±0 (0/12)	0±0 (0/12)	0±0 (0/12)
Erosions/rupture-associated thrombosis					
Vehicle (n=8)	0±0 (0/8)	0±0 (0/8)	0±0 (0/8)	0±0 (0/8)	0±0 (0/8)
Ang II-L (n=10)	1.0±1.25 (5/10)	0.6±0.52* (6/10)	0.5±0.97 (3/10)	0±0 (0/10)	0±0 (0/10)
Ang II-H (n=12)	1.33±1.07** (9/12)	1.0±1.04* (7/12)	0.73±0.65* (7/12)	0±0 (0/12)	0±0 (0/12)

The number of each type of lesions was calculated from all sections of each block. The largest number on the section was used to represent each block. The data are expressed as the mean±SD. Parentheses indicate the ratio of animals with the lesions to total animal number. Thrombosis includes both partial and occluded thrombi. Ang II indicates angiotensin II.

*$P<0.05$, **$P<0.01$ vs vehicle group using the Mann–Whitney U test.

In Experiment 2, WHHL rabbits were first infused with relatively low doses of Ang II for 4 weeks. In contrast to Expt 1, except for 1 rabbit that died at 2 weeks, all rabbits survived until 4 weeks and further received high doses of Ang II for another 4 weeks (see Fig. 11). This resulted in deaths of 5/7 (71%) rabbits (Fig. 14). No deaths occurred in the vehicle group. As in Expt 1, the levels of blood pressure (both groups) and blood leukocytes (high-Ang II group) were significantly higher in the Ang II groups than in the vehicle group (Fig. 15). Similar to the Expt 1, pulmonary edema/hemorrhage and MI were present in all dead rabbits. Similarly, coronary plaque rupture/erosion with thrombosis was present in Ang II groups (Table 10), but coronary stenosis of the Ang II groups was not significantly different from the vehicle group.

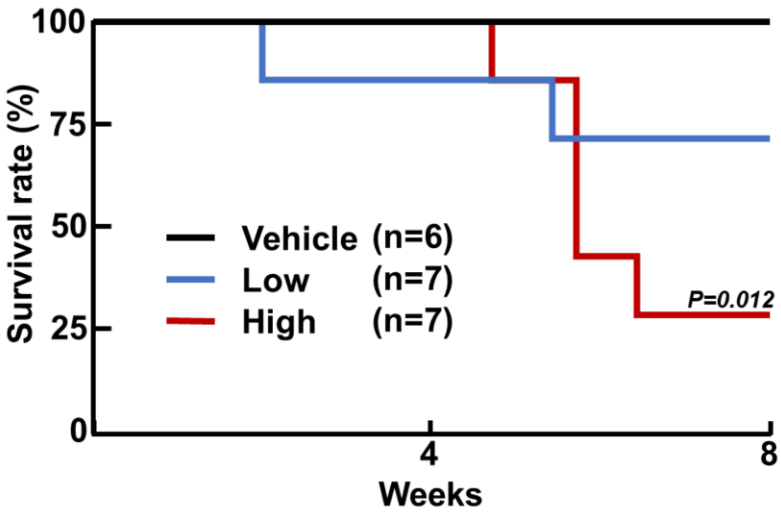

Figure 14. Experiment 2. Kaplan–Meier analysis of cumulative rates of survival in WHHL rabbits after Ang II slow infusion.

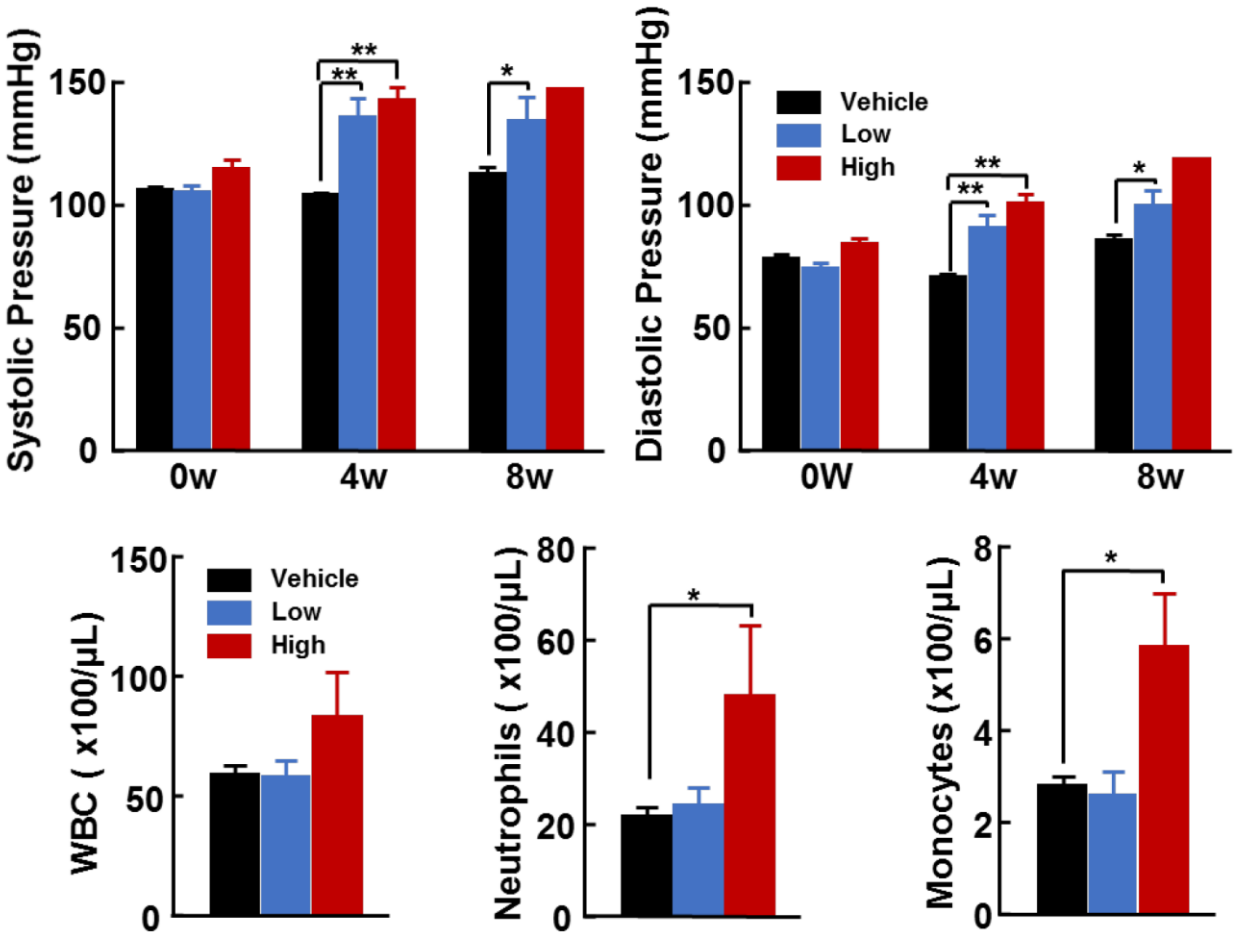

Figure 15. Blood pressure (upper) at 0, 4 and 8 weeks, and blood leukocyte count (lower panel) at 6 weeks were measured after Ang II infusion.

Data are expressed as mean ± SEM. *P<0.05 or **P<0.01 versus the vehicle. n=6 for the vehicle, n=5-7 for low-Ang II and n=1-7 for high-Ang II groups.

Table 10. Experiment 2. Number of coronary erosion, rupture, and thrombosis in each block

Erosions

	Block I	Block II	Block III	Block IV	Block V
Vehicle	0.333 ± 0.5	0.33 ± 0.52	0 ± 0	0 ± 0	0 ± 0
(n=6)	(2/6)	(2/6)	(0/6)	(0/6)	(0/6)
Ang II-L	1.142 ± 0.69	1.0 ± 0.58	0 ± 0	0 ± 0	0 ± 0
(n=7)	(6/7)	(6/7)	(0/7)	(0/7)	(0/7)
Ang II-H	0.71 ± 0.76	0.57 ± 0.53	0 ± 0	0 ± 0	0 ± 0
(n=7)	(4/7)	(4/7)	(0/7)	(0/7)	(0/7)

Ruptures

	Block I	Block II	Block III	Block IV	Block V
Vehicle	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0
(n=6)	(0/6)	(0/6)	(0/6)	(0/6)	(0/6)
Ang II-L	0.428 ± 0.79	0 ± 0	0 ± 0	0 ± 0	0 ± 0
(n=7)	(2/7)	(0/7)	(0/7)	(0/7)	(0/7)
Ang II-H	0.71 ± 0.49*	0.14 ± 0.38	0 ± 0	0 ± 0	0 ± 0
(n=7)	(5/7)	(1/7)	(0/7)	(0/7)	(0/7)

Erosions/rupture-associated thrombosis

	Block I	Block II	Block III	Block IV	Block V
Vehicle	0 ± 0	0.17 ± 0.41	0 ± 0	0 ± 0	0 ± 0
(n=6)	(0/6)	(1/6)	(0/6)	(0/6)	(0/6)
Ang II-L	1.0 ± 1.0	1.0 ± 0.58*	0 ± 0	0 ± 0	0 ± 0
(n=7)	(4/7)	(6/7)	(0/7)	(0/7)	(0/7)
Ang II-H	1.14 ± 0.69**	0.71 ± 0.49	0 ± 0	0 ± 0	0 ± 0
(n=7)	(6/7)	(5/7)	(0/7)	(0/7)	(0/7)

The number of each type of lesions was calculated from all sections of each block. The largest number on the section was used to represent each block. The data are expressed as the mean ± SD. *P<0.05, **P<0.01 vs vehicle group using the Mann-Whitney U test. Parentheses indicate the ratio of animals with the lesions to total animal number. Thrombosis includes both partial and occluded thrombi.

Microscopic examinations showed that luminal surface macrophages of the aortic arch were increased in Ang II-infused groups. Quantification by the real-time polymerase

chain reaction (RT-PCR) showed significantly ($p < 0.05$) higher mRNA expression of interleukin-6 (IL-6) and plasminogen activator inhibitor-1 (PAI-1) in aortic lesions in rabbits receiving Ang than the vehicle group. Expression of monocyte chemoattractant protein-1 (MCP-1), IL-1β, tumor necrosis factor-α (TNF-α), and MMP-9 were also increased, whereas expression of collagens I and III were reduced relative to those in the vehicle group (Fig. 16). These results suggest that chronic Ang II exposure provokes vascular inflammation and thrombosis.

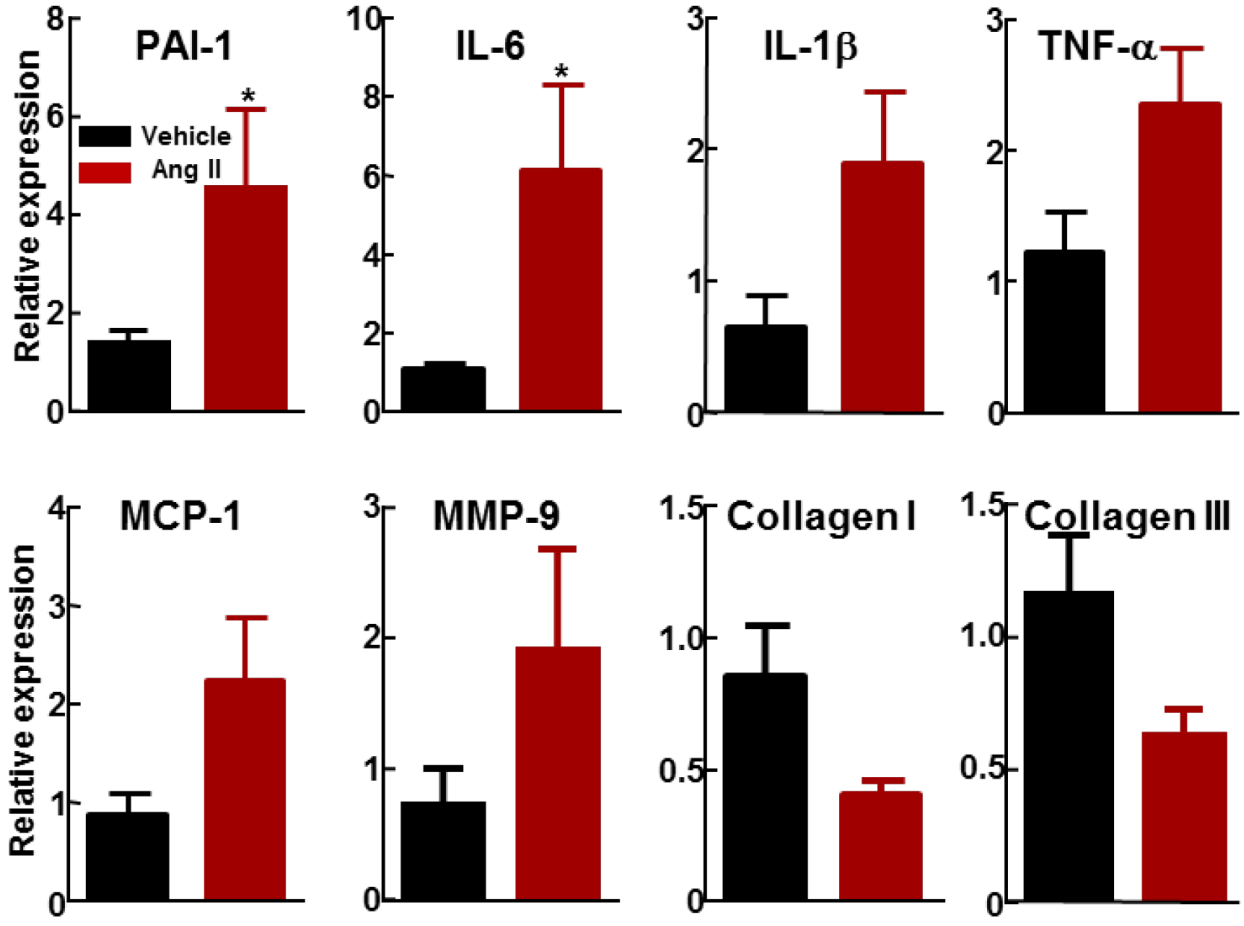

Figure 16. RT-PCR analysis of mRNA expression of aortic lesions of the aortic arch.

RT-PCR was used to quantify each gene expression. IL-1β = interleukin-1β; IL-6 = interleukin-6; MCP-1 = monocyte chemoattractant protein-1; MMP9 = matrix metalloproteinase-9; PAI-1 = plasminogen activator inhibitor-1; TNF-α = tumor necrosis factor-α. N = 5 for each group; *$p < 0.05$ vs vehicle group

In summary, the study demonstrated that infusion of Ang II into Watanabe heritable hyperlipidemic rabbits, induced coronary complications, thrombosis, myocardial infarction and deaths.

7 Genetic Toxicology

No genotoxicity studies have been conducted by the sponsor on LJPC-501 as Ang II is a naturally occurring peptide in the body.

On PubMed search by this reviewer, several publications were found on the genotoxic potential of Ang II and aldosterone, the main components of RAAS that are elevated in hypertension. The findings from a few selected research papers are summarized.

a) Brand S, et al. Angiotensin II-induced hypertension dose-dependently leads to oxidative stress and DNA damage in mouse kidneys and hearts. *J Hyperten,* **31**: 333–344 (2013).

   Male C57BL/6-mice were randomized into 5 different groups (n=8/group) and were subcutaneously implanted with osmotic mini pump. Ang II in four different concentrations, 60 (No-Pressor), 200, (Slow-Pressor 1), 400 (Slow-Pressor 2) and 1000 ng/kg/min (Pressor) was delivered for 28 days. Control (group 1) animals received the solvent PBS. After 4 weeks of treatment, animals were sacrificed and kidneys and the heart were removed for histopathology.

   Ang II infusion rapidly led to a significant increase of the systolic blood pressure, which stayed high until the end of the experiment, and was significantly higher than the control values at all days except on days 10 and 14. Significant blood pressure changes were absent in animals infused with the low concentration (60 ng/kg/min) of Ang II. Ang II-induced high BP resulted in severe histopathological changes in the kidney. Glomeruli of control mice showed normal architecture, whereas glomeruli of Pressor group mice showed an expansion of mesangial matrices, focal sclerosis and moderate capillary widening. Glomerular sclerosis index and mesangiolysis index dose-dependently increased within the four different treatment groups. Tubulointerstitial injury of Pressor group mice was predominantly driven by tubular dilatation and interstitial fibrosis, whereas inflammation was prevalent in only a few mice. Tubulointerstitial sclerosis index was fourfold higher in the Pressor group relative to control group. Vascular injury, expressed as thickening of vessel walls, also increased dose-dependently.

   The oxidative capacity of Ang II was tested in vivo with the cryosections of kidneys and hearts of mice of all groups. The tissues were stained with the ROS-sensitive dye dihydroethidium, which mainly detects superoxide. Quantification of the fluorescence revealed a dose-dependent increase (statistically significant starting in the Slow-Pressor 1 group) of ROS production in the kidney (up to 1.7-fold over control). In the heart, only in the Pressor group, statistical significance (1.4-fold over control) was reached. To analyze Ang II-caused structural DNA damage in the tissue, paraffin sections were immunostained with an antibody against the DNA double-strand break marker γ-H2AX (phosphorylated histone H2A family). Tubulus cells positive for γ-H2AX were more abundant in kidneys of the Pressor group. Quantification of γ-H2AX-positive tubulus cells showed a clear

Ang II dose-dependency of the formation of DNA strand breaks (up to 4.7-fold over control). Remarkably, the No-Pressor group (60 ng/kg/min) showed a trend to increased DNA damage. In the heart tissue, cells positive for γ-H2AX were observed (13-fold over control in the high dose group). Ang II also induced formation of 7,8-dihydro-8-oxo-guanine (8-oxodG), the mutagenic DNA base modification or lesion. Kidney DNA revealed a dose-dependent (up to 3.6-fold over control) increase in mutagenic DNA lesions, which was statistically significant in the Slow-Pressor 2 and Pressor groups. Administration of tempol (radical scavenger) significantly decreased (20%) oxidative stress in the kidney and DNA double-strand breaks in the kidney (60%) and in the heart (52%) without lowering the blood pressure.

In summary, the authors showed oxidative stress mediated genotoxic effects of Ang II in vivo. Furthermore, the increase of 8-oxodG suggests a mutagenic potential of an activated renin–angiotensin–aldosterone system, which is often found in hypertensive patients.

b) Brand S, et al. Oxidative DNA damage in kidneys and heart of hypertensive mice is prevented by blocking Angiotensin II and aldosterone receptors. *PLoS ONE,* **9(12)**: e115715 (2014). doi:10.1371/journal.pone.0115715.

This is an extension of the above study by the same authors wherein they have investigated the mechanism of DNA damage caused by Ang II, and the possible intervention strategies against end-organ damage, that is the effects of substances interfering with the renin-angiotensin-aldosterone-system on Ang II-induced genomic damage.

As in the above study, hypertension was induced in male C57BL/6-mice by infusion of 600 ng/kg/min Ang II by subcutaneously implanted mini osmotic pump. The animals were additionally treated with the candesartan (Ang II AT-1 receptor blocker), eplerenone (the mineralocorticoid receptor blocker) and tempol (the antioxidant). DNA damage and the activation of transcription factors were studied by immunohistochemistry and protein expression analysis.

Ang II caused a significant increase in blood pressure and was reduced only by candesartan. In kidneys and hearts of Ang II treated animals, significant oxidative stress could be detected (1.5-fold over control). In the kidney, Ang II-treatment activated the antioxidative defense, Nrf2 (nuclear factor erythroid 2-related factor 2), resulting in the translocation to the nucleus and the expression of antioxidative proteins. In addition, Ang II treatment activated proinflammatory transcription factor NF-kB, its translocation to the nucleus and subsequently to the expression of NF-kB target genes. Both these activations were reduced by all 3 interventions. In kidneys and hearts, an increase in DNA damage (3- and 2-fold over control, respectively) and of DNA repair (3-fold over control) was found. These effects were ameliorated by all interventions in both organs. Consistently,

candesartan and tempol were more effective than eplerenone. In conclusion, Ang II-induced DNA damage is caused by AT-1 receptor-mediated formation of oxidative stress in vivo. The Ang II-mediated physiological increase of aldosterone adds to the DNA-damaging effects. Blocking Ang II and mineralocorticoid receptors therefore has beneficial effects on end-organ damage independent of blood pressure normalization.

c) Bianca P, et al. Renovascular hypertension leads to DNA damage and apoptosis in bone marrow cells. *DNA and Cell Biology,* **32**:458-466 (2013). https://doi.org/10.1089/dna.2013.2065

Angiotensin II (Ang II), which plays a pivotal role in the pathophysiology of the two-kidney, one-clip (2K1C) Goldblatt hypertension, has been associated with augmented generation of reactive oxygen species (ROS) in some cells and tissues. In the present study, the authors evaluated the influence of 2K1C hypertension on oxidative stress, DNA fragmentation, and apoptosis of bone marrow (BM) cells. Two weeks after the renal artery clipping or Sham operation, flow cytometry analysis showed a higher production of superoxide anions (approximately six-fold) and hydrogen peroxide (approximately two-fold) in 2K1C hypertensive than in Sham normotensive mice. 2K1C mice also showed an augmented DNA fragmentation (54%) and apoptotic cells (21%). The data show that the 2K1C renovascular hypertension is characterized by an increased production of ROS, DNA damage, and apoptosis of BM, which is a fundamental source of the cells involved in tissue repair.

d) Fazeli G, et al. Angiotensin II induces DNA damage via AT1 receptor and NADPH oxidase isoform Nox4. *Mutagenesis,* **27**:673–681 (2012).

Epidemiological studies reveal increased renal cancer incidences and higher cancer mortalities in hypertensive individuals. Activation of the RAAS leads to the formation of reactive oxygen species (ROS). In vitro, in renal cells, and ex vivo, in the isolated perfused mouse kidney, the authors show the DNA-damaging potential of Ang II. Here, the pathway involved in the genotoxicity of Ang II was investigated.

In kidney cell lines with properties of proximal tubulus cells, an activation of NADPH oxidase and the production of ROS, resulted in the formation of DNA strand breaks and micronuclei induction. This DNA damage was mediated by the AT1R, together with the G protein Gα-q/11. Subsequently, phospholipase C (PLC) was activated and intracellular calcium increased. Both calcium stores of the endoplasmic reticulum and extracellular calcium were involved in the genotoxicity of Ang II. Downstream, a role for protein kinase C (PKC) could be detected, because its inhibition hindered Ang II from damaging the cells. Although PKC was activated, no involvement of its known target, the NADPH oxidase isoform containing the Nox2 subunit, could be found, as tested by small-

interfering RNA downregulation. Responsible for the DNA-damaging activity of Ang II was the NADPH oxidase isoform containing the Nox4 subunit.

In summary, in kidney cells the DNA-damaging activity of Ang II depends on an AT1R-mediated activation of NADPH oxidase via PLC, PKC and calcium signaling, with the NADPH subunit Nox4 playing a crucial role.

e) Zimnol A, et al. Angiotensin II type 1a receptor-deficient mice develop angiotensin II-induced oxidative stress and DNA damage without blood pressure increase. *Am J Physiol Renal Physiol* (September 6, 2017). doi:10.1152/ajprenal.00183.2017

Hypertensive patients have an increased risk to develop kidney cancer. The authors have shown in vivo that besides elevating blood pressure, Ang II causes a dose-dependent damage to DNA. Here, the role of blood pressure in the formation of DNA damage was studied.

Mice lacking one of the two murine angiotensin II type 1 receptor subtypes, AT1aR, were equipped with osmotic minipumps, delivering angiotensin II for 28 days. Parameters for oxidative stress and DNA damage in kidneys and hearts of AT1aR-knockout mice were compared to wildtype (C57BL/6) mice receiving angiotensin II, and additionally, to wildtype (WT) mice treated with candesartan, an antagonist of both AT1R-subtypes.

In WT mice, angiotensin II induced hypertension, reduced kidney function and led to a significant formation of reactive oxygen species (ROS). Furthermore, genomic damage was markedly increased in this group. All these responses to angiotensin II could be attenuated by concurrent administration of candesartan. In AT1aR-deficient mice, infusion of angiotensin II did not increase systolic pressure and did not affect renal function. However, in knockout animals, angiotensin II still increased ROS in kidneys and hearts. Additionally, genomic damage in the form of double-strand breaks, was significantly induced in kidneys of AT1aR-deficient mice. These results show that angiotensin II-induced ROS production and subsequent DNA damage occur even in the absence of AT1aR and independent of blood pressure changes.

In summary, beyond adverse organ effects such as cardiac hypertrophy and albuminuria, Ang II independent of blood pressure promotes inflammatory responses, cellular growth and oxidative stress. We can now add genomic damage to this list of blood pressure-independent effects. Since DNA damage seems to have a critical role in the development of chronic diseases such as cancer, it might be interesting to further evaluate antihypertensive therapies for their potential to prevent the accumulation of DNA lesions and for the effect on the cancer risk in this patient group.

8 Carcinogenicity

No studies have been conducted by the sponsor as LJPC-501 is intended for short duration. However, a literature search (listed below and are referenced in this section by numbers) by this reviewer found increased cancer incidences in hypertensive patients (1,2). In most hypertensive patients, a stimulated RAAS is present as increased Ang II concentrations contribute to multitude of disease, including hypertension, atherosclerosis, kidney disease and possibly cancer (3-5). The renin angiotensin system has links to cancer through tissue remodeling, inflammation, angiogenesis and apoptosis (5-7). In vitro, animal and clinical studies indicate that the RAS is frequently dysregulated in malignancy and correlates with poor patient outcomes (8).

The vasoconstrictor, salt and water balance actions of Ang II are mediated by AT-1 receptors that are coupled to Gq/11. Additionally, the activated AT-1 receptor is coupled to MAPKs, JAK-STAT pathway, tyrosine kinases, receptor tyrosine kinases (i.e., EGFR, PDGF, insulin receptor), nuclear factor κB and reactive oxygen species (ROS) (7). The pro-inflammatory and pro-angiogenic signaling of the Ang II is the result of generation of ROS through cell-type specific NADPH oxidases (reviewed in reference 7), which activate downstream signaling cascades, including the activation of the MAPK and PI3K–Akt pathways and other redoxsensitive transcriptional factors. The excessive accumulation of ROS, leads to oxidative stress, followed by increased protein modifications, cellular damage and death or increased growth factor signaling (reviewed in reference #9). Oxidative stress coupled with increased expression of the inflammatory marker inducible nitric oxide synthase increases 8-hydroxy-2'-deoxyguanosine, a marker of DNA damage (10). RAAS inhibitors mostly suppresses tumor growth, metastasis and angiogenesis in a broad range of experimental models of malignancy (8,11). Thus, these drugs have broad clinical utility and have shown to have a decreased risk of developing some types of cancers (12-14).

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9 Reproductive and Developmental Toxicology

No studies are included in the original submission. On our request, the sponsor submitted a comprehensive literature search on the topic. Extensive studies have been done linking Ang II or Ang II signaling to fertility or reproduction in humans and animals. Any effects with Ang II may be transient and reversible given that its half-life is less than a minute. There is no information on the effect of Ang II on lactation in women. Here we summarize findings from a few clinical and nonclinical studies (publications listed below are referenced by numbers in the text) conducted with Ang II.

Fertility: On isolated human sperm, Ang II (0.3 to 100 mM) has a potential fertility-stimulating effects on sperm maturation. It stimulates sperm motility, including curvilinear velocity, straight line velocity, and amplitude of lateral head movement (1). The effects are mediated through AT-1 receptors as they are localized in the head and tail of the capacitated sperm, and are localized in the tail only in noncapacitated sperm (2). Ang II added to semen during artificial insemination in cattle, increased the retention of spermatozoa within the uterus of heifers (young cows) through AT-1 receptors mediated stimulation of myometrial contraction (3).

In human males, Ang II levels in the penile corpus cavernous plasma significantly increase from the flaccidity phase to the detumescence phase, contributing toward contraction (4).

In rabbit ovaries perfused in vitro, Ang II (0.1 to 100 ng/ml) induced ovulation and meiotic maturation of oocytes at all concentrations. This effect is mediated by AT-2 receptors (5,6). Ang II improves oocyte cytoplasmic maturation, resulting in a higher rate of embryo development (7). In primary cultures of human endometrial stromal cells, Ang II through AT-1 receptors stimulated cell proliferation, up-regulated the expression of smooth muscle actin (SMA), transforming growth factor β1 (TGF-β1), and insulin-like growth factor (IGF-I), and increased the secretion of extracellular matrix (type I collagen and fibronectin). These results suggest Ang II may contribute to endometriosis and that Ang-(1-7) plays a counter-regulatory role (8).

Pregnancy: As discussed in an earlier section (4.2.4), plasminogen is the key component of the fibrinolytic system that plays a role in the digestion of fibrin clots. Plasminogen activator inhibitor-1 (PAI-1) inhibits fibrinolysis and in addition, controls cell

migration, invasion in tumor growth and angiogenesis, and placental development. Studies on a human trophoblasts cell line showed that Ang II through the activation of AT-1 receptor stimulated PAI-1 synthesis and secretion in a time- and concentration-dependent manner (9). In pre-eclampsia, elevated PAI-1 occurs in the maternal circulation as circulating Ang II levels are highest and this has been implicated as a contributing risk factor for hypercoagulation and fibrinolytic imbalance (see also section 4.2.4). The study also suggest that the upregulated local RAS plays an important role in normal placental development. Women who eventually develop preeclampsia become hyper-responsive to the pressor effects of Ang II in late pregnancy and postpartum. Ang II responsiveness may remain elevated in this group, particularly among women with low sodium balance, reduced plasma volume and/or evidence of other underlying disorders (10-12). It is suggested that pressor sensitivity to exogenous Ang II is decreased in normal pregnancy, but restored postpartum (13,14).

Pregnant rats exposed to Ang II via osmotic pumps from gestation days 6 to 21 had no effect on the litter size, birth weight and sex ratio of the pups (15). Additionally, no treatment effects were seen on survival, growth (body weight and body weight gain), reproductive performance/fertility, parturition, F1 litter data, or organ weights (heart and kidneys). However, increased Ang II during pregnancy raised BP in the offspring, and salt sensitivity was decreased relative to controls. The results suggest that conditions leading to upregulation of RAAS during pregnancy can influence the cardiovascular system of the fetus and have a long-term impact on the offspring health. An increase in fetal arterial blood pressure was also noted in fetal sheep when Ang II was infused into the fetal femoral artery of pregnant ewes (gestational age 120-136 days). Ang II caused a greater increase in arterial blood pressure when infused for 3 or 5 days (16). In guinea pigs, administration of Ang II in mid pregnancy (from gestation days 20 to 34) resulted in high proportions of aborted and dead fetuses suggesting Ang II impairs gestational outcome (17). In a study of normotensive pregnant women (24-38 weeks gestation) with placental vascular disease identified by Doppler, Cook and Trudinger (1991) (18) found a correlation between a positive Ang II pressor response (>20 mm Hg increase in DBP with <20 ng/kg/min Ang II) and early labor, fetal distress, and low birth weight.

Pre- and Postnatal Development: Intravenous infusion of Ang II to rats from gestational day 17 through lactation day 21 produced no maternal toxicity. Beyond lactation day 21 (postweaning), Ang II had no effect on the reproductive function of F1 pups except for decreases in absolute and relative kidney, heart, liver and brain weights. No lethality, development or teratogenic effects were observed on the fetus (F2 generation) (19). Similar observations (i.e., no effect on F1 generation) were made by Svitok et al. (2017) (15) in pregnant rats receiving Ang II from gestation day 6 through gestation day 21.

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10 Integrated Summary and Safety Evaluation

Ang II, the primary effector hormone of the RAAS, acts on AT-1 and AT-2 receptors. The receptors are members of the G protein-coupled receptor superfamily. Both subtypes are selectively antagonized by respective class antagonists. Activation of the AT-1 receptor (coupled to Gq/11) regulates cardiovascular and renal physiology manifested as control of blood pressure, aldosterone secretion, renal sodium absorption and water intake. Ang II through AT-1 receptors stimulates multiple signaling pathways (both G protein-dependent and G protein-independent), cross-talks with several tyrosine kinases, and transactivates growth factor receptors (reviewed by Balakumar and Jagadeesh 2014, #19). This results in an array of physiologic and toxicologic effects that are initiated and sustained based on the duration and concentrations of Ang II in plasma and tissue. On the other hand, AT-2 receptor activation promotes different and often opposing effects to those of AT-1 receptors as it causes vasodilation (by increasing the production of nitric oxide and cGMP), antiproliferation, apoptosis and inhibition of cell growth, cardiac fibrosis and cardiomyocyte growth (18).

However, the sponsor notes that the main objective in the clinical use of LJPC-501, is to restore systemic blood pressure from the hypotensive to normotensive state, and not to induce a hypertensive state. The duration of infusion of LJPC-501 is for a limited period.

Pharmacology

Pharmacodynamics
LJPC-501 is a synthetic human angiotensin II. In vitro receptor binding and functional activity studies have demonstrated similar potency and comparable activity between LJPC-501 and alternative forms of Ang II synthesized based on the bovine (Hypertensin®, manufactured by Ciba-Geigy Pharmaceutical Co) or human (multiple manufacturers, including (b) (4) etc) sequence. Pharmacology studies of Ang II relevant to the proposed indication, to treat hypotension in adults with distributive shock who remain hypotensive despite fluid and vasopressor therapy, and the intended intravenous route of administration have been reported in the literature.

In animal models of hypotensive and hyperdynamic sepsis, intravenous Ang II infusion restored arterial pressure and decreased renal blood flow while maintaining renal perfusion and improved glomerular filtration, urinary output and sodium excretion. Also, normalized creatinine clearance suggesting improved kidney function. Any diminished reactivity to vasoconstrictors during sepsis is the result of enhanced production of endogenous proinflammatory mediators such as TNF-α, IL-1β, IFN-γ, and IL-6. Septic shock is characterized by systemic vasodilation with decreased reactivity to vasoconstrictors, leading to arterial hypotension, multiple organ dysfunction and death. Septic shock induced in animals with live intravenous E. coli demonstrated sustained fall in MAP accompanied by marked renal vasodilation and a decrease in renal blood flow. Treatment with Ang II restored MAP to baseline levels with significantly reduced

renal vascular conductance. Additionally, Ang II during treatment of shock did not cause kidney ischemia/damage. In another published study, Ang II reversed sepsis-induced hypotension in pigs, sheeps with systemic and regional hemodynamic effects similar to those of norepinephrine. Inhibition of Ang II (by ACEi or ARB) exacerbated anaphylaxis-induced hypotension in anesthetized rats.

Secondary pharmacology
Ang II affects glucose control via stimulation of gluconeogenesis and impairment of insulin sensitivity. In rats, IV administration of Ang II was accompanied by an activation of gluconeogenesis, as evidenced by a rapid and marked increase in the rate of incorporation of ^{14}C from [^{14}C]-bicarbonate into circulating glucose resulting in hyperglycemia. Thus, Ang II is involved in the pathogenesis of both hypertension and insulin resistance. The mechanism appears to involve increased oxidative stress, possibly via impaired insulin signaling to a point downstream of PI 3-kinase activation. These findings strongly suggest oxidative stress causes insulin resistance with hypertension (17).

Safety pharmacology
Ang II produces an inhibitory effect on IKr/hERG currents via AT-1 receptors linked to the PKC pathway in ventricular myocytes. This is a potential mechanism by which elevated levels of Ang II are involved in the occurrence of arrhythmias in cardiac hypertrophy and failure. A GLP hERG study was conducted to demonstrate the effect of high (60 μM) concentrations of LJPC-501 on the delayed rectifier K+ current. No effect of angiotensin II on IKr (hERG) currents was found in HEK293 cells unless AT1 receptors were co-expressed. In α-chloralose anesthetized dogs, LJPC-501 elicited ($p < 0.05$) dose-dependent (≥450 ng/kg) elevations in mean arterial pressure and systemic vascular resistance. At the highest dose (1800 ng/kg), LJPC-501 elicited statistically significant elevations in left ventricular systolic pressure, and left ventricular end diastolic pressure, heart rate as well as a non-statistically significant increase in PR interval. No relevant effects were observed for the QT/QTcV interval or respiratory rate at any tested dose of LJPC-501. At the lowest dose assessed (150 ng/kg), LJPC-501 did not elicit any statistically significant or physiologically relevant hemodynamic, cardiac function, ECG or respiratory effects.

Several published both animal and clinical studies suggest that Ang II is likely to contribute to prothrombotic state by reducing the activity of the endogenous fibrinolysis system. Physiologically, Ang II counteracts the loss of intravascular volume by inducing vasoconstriction, and retention of salt and water. In this process, it is logical that Ang II possesses intrinsic hemostatic effects that promote thrombosis and reduce fibrinolysis by activation of platelet function and plasminogen activator inhibitor-1. These mechanisms help Ang II to restore and preserve vascular integrity after injury (22).

Pharmacokinetics

The PK profile of exogenously administered Ang II in animals and humans are reported extensively in the literature. Ang II produces dose-proportional pressor response and systemic exposures over a wide range of dose levels. About 47% to 76% of infused Ang II is cleared in one circulation in all vascular beds giving an estimated half-life of approximately 15 to 16 sec in dogs and rats, 45 sec in sheep, and <60 sec in humans. Ang II has a high blood clearance rate with relative volume of distribution with a higher concentration in arterial plasma than in venous plasma. Plasma protein binding is approximately 50%. Ang II (1-8 peptide) is rapidly metabolized at both the amino and carboxy termini into pharmacologically active Ang III (2-8 peptide), Ang IV (3-8 peptide) and Ang 1-7, and other inactive smaller peptide fragments in a variety of tissues. Thus, insignificant amount is excreted in the urine.

Toxicology

The published literature provides a comprehensive understanding of the toxicologic properties of Ang II administered intravenously or subcutaneously (e.g., by miniature osmotic pumps) in animals. The toxicities of exogenously administered Ang II are manifested as excessive pressor effects on the vasculature, heart and kidney, the target organs of action. The effects of Ang II are dose- and duration-dependent. Ang II showed pronounced effect on mean arterial blood pressure that was greater in normotensive than in hypertensive animals.

In normotensive rabbits, low dose (10 ng/kg/min) intravenous administration of Ang II for 14 days caused an increase in blood pressure (+53 mm Hg) but there was no evidence of vascular or cardiac toxicity (Hu et al. 2004). In contrast, single intravenous injection of very large doses (0.5 µg/kg or 100 µg/rat) of Ang II caused acute medial necrosis of large renal arteries in the rat. The necrosis appears to be an immediate result, direct or indirect, of the double physical stress imposed on the arteries by overstimulation and excessive filling tension. Repeated doses of Ang II up to 7 hr occasionally caused focal glomerular "necroses", which are derived from capillary aneurysms by a process of thrombosis. In rats with chronic unilateral renal hypertension similarly treated with Ang II, both arterial and glomerular lesions were absent from the intact kidney, but present and often severe in the "clipped" kidney (23). Similar results were demonstrated in rats and rabbits, wherein large doses (>100 ng/kg/min) of Ang II infused for 24 hr or long produced extensive myocardial and renal injuries including widespread focal myocardial infarction, acute renal failure with uremia and renal tubular necrosis (24-29). The severity of the renal and cardiac lesions is closely correlated to pressor effects and the elevation (2- to 3-fold normal values) of arterial plasma Ang II levels. In the rabbit, the high circulating concentrations of Ang II, which is 2 to 3 times the normal, is associated with systemic hypertension, and this in turn causes myocardial and kidney necroses.

A close interrelationship appears to exist between both the pressor and cytotoxic effects of Ang II and nitric oxide blockade. During chronic NOS inhibition, co-infusion of low

dose of Ang II (4 ng/kg/min) was shown to have an additive effect on pressor response. Rapid blood pressure increases result in swift and extensive damage to heart and kidneys. This does not occur when L-NAME (NOS inhibitor) or Ang II is administered alone over the same time frame. These observations suggest that under conditions of reduced NO bioavailability (e.g., endothelial insufficiency) even small increases in Ang II may result in target organ damage (26,27,29).

A preponderance of mortality and cardiovascular toxicity was reported in hyperlipidemic rabbits with chronic infusion of Ang II (30). Rabbits received Ang II either rapidly (100 or 200 ng/kg/min for 4 weeks) or gradually (increase from 50 or 75 ng/kg/min in the first 4 weeks to 100 or 150 ng/kg/min for an additional 4 weeks) showed high mortality with increased blood pressure followed by multiple local effects on the arterial wall triggered coronary plaque erosion, thrombosis and subsequent MI. The study showed that Ang II affects several functions of the arterial wall cells, such as endothelial cells, smooth muscle cells, and macrophages, thereby provoking vascular inflammation. Increased expression of proinflammatory cytokines, PAI-1 and MMPs, and reduction in the expression of endothelial NOS together facilitate the transformation of a stable plaque into a vulnerable plaque prone to rupture. Another possibility for Ang II–induced plaque rupture is associated with the fact that Ang II is notorious for inducing vascular oxidative stress because of the generation of reactive oxygen species, which plays an important role in the pathogenesis of atherosclerosis (31)

Genotoxicity

In vitro studies have demonstrated genotoxic potential of both Ang II and aldosterone (32,33). Both cause DNA single and double strand breaks, abasic sites and increase the abundance of the DNA base modification, 7,8-dihydro-8-oxo-guanine. In experimental hypertension, either caused by infusion of Ang II or aldosterone, DNA lesions could be found in kidneys and heart of animals (34-36). The underlying mechanism is the activation of ROS generating enzymes like NADPH oxidase, via either the AT-1 receptor or the mineralocorticoid receptor (35-37).

Reproductive toxicity

Ang II has no adverse effect on male or female reproductive organs, fertility, organogenesis, or fetus development, survival and birth. Ang II thru AT-2 receptors induces ovulation and meiotic maturation of oocytes and favors a higher rate of embryo development. Increased Ang II during pregnancy raises BP in the offspring, salt sensitivity is decreased relative to controls and have a long-term impact on the offspring health. A correlation exists between a positive Ang II pressor response and early labor, fetal distress, and low birth weight.

In pre-eclampsia, elevated plasminogen activator inhibitor-1 occurs in the maternal circulation as circulating Ang II levels are highest and this has been implicated as a contributing risk factor for hypercoagulation and fibrinolytic imbalance. Upregulated RAS

plays an important role in normal placental development. Women who eventually develop preeclampsia become hyper-responsive to the pressor effects of Ang II in late pregnancy and postpartum. Ang II responsiveness may remain elevated in this group, particularly among women with low sodium balance, reduced plasma volume and/or evidence of other underlying disorders. Pressor sensitivity to exogenous Ang II is decreased in normal pregnancy, but restored postpartum.

Evaluation

LJPC-501 is a synthetic human Ang II that has been studied extensively in various species including humans since the 1950s. The current drug product is the same form of Ang II that was used in many studies and manufactured by several companies. Primary pharmacodynamic studies involving animal models of septic shock demonstrated therapeutic benefit of LJPC-501 in humans with hypotensive shock, the proposed indication of the product.

Ang II is a natural vasopressor hormone of the body that regulates cardiovascular and renal physiology. However, it is not devoid of serious adverse and toxic effects when its circulating concentration exceeds physiological levels (5 to 20 pg/ml). High circulating concentrations of Ang II are associated with systemic hypertension, and this in turn causes myocardial and kidney necroses. The toxicities of exogenously administered Ang II is manifested as excessive pressor effects on the vasculature, heart and kidney, the target organs of action. The effects of Ang II are dose- and duration-dependent.

Ang II mediates many of its effects by binding to two major G protein-coupled receptors AT-1 and AT-2. The receptor subtypes have different growth and development, health and disease patterns and are distributed in varying proportions in different tissue beds. Activation of the AT-1 receptor increases arterial blood pressure by a direct vasoconstrictor action on blood vessels and promotes renal sodium reabsorption and water retention by stimulating adrenal aldosterone secretion. Activation of AT-1 receptors transduces G protein-dependent and G protein-independent signals, cross talks with several tyrosine kinases, and transactivate growth factor receptors resulting in cardiac and renal disorders, endothelial dysfunction, cell survival, migration of cells, hypertrophy, cardiac fibrosis, glomerulosclerosis, proteinuria, thrombosis and DNA damage. The latter activity of Ang II is the result of AT-1 receptor activation of NADPH oxidases and generation of ROS. DNA damage seems to have a critical role in the development of chronic diseases such as cancer (38). Ang II possesses intrinsic hemostatic defects that promote thrombosis and reduce fibrinolysis by activation of platelet function and PAI-1. Increased thrombosis is associated with preeclampsia as circulating Ang II levels are highest. Women who develop preeclampsia become hyper-responsive to the pressor effects of Ang II in late pregnancy and postpartum (39).

Angiotensin II activated AT-1 receptor mediated harmful effects are to some extent opposed by AT-2 receptors that induce vasorelaxation and promotion of natriuresis. The AT-2 receptor system appears to provide an endogenous protection to inflammatory, oxidative and apoptotic processes (18). However, it is not clear why AT-2 receptors

cannot adequately or efficiently antagonize the vascular and cardiac effects of AT-1 receptors in both physiologic and pathologic conditions. Some of the reasons are the threshold concentration of Ang II needed for response is much higher for the AT-2 receptor than for the AT-1 receptor. Plasma Ang II rather than tissue Ang II is the agonist of the AT-2 receptor, and the reverse applies to the AT-1 receptor. Thus, the AT-2 receptor stimulation may come into play only at unusually high circulating levels of Ang II (16). In addition, distribution, density and location of receptors in tissues determines the AT-1 receptor has strong competition with the AT-2 receptor in cardiovascular control of Ang II.

All the above deleterious effects of Ang II that occur with excess stimulation of the AT-1 receptor may not arise with the current product if therapeutic doses are not exceeded. The main objective in the clinical use of LJPC-501, is to restore systemic blood pressure from the hypotensive to normotensive state, and not to induce a hypertensive state or restrict tissue blood flow. According to the sponsor, any excessive pharmacologic and toxicologic effects of exogenously administered Ang II on the vasculature, heart and kidney observed in normotensive subjects is unlikely to occur in patients with distributive or vasodilatory shock if titrated to lowest dose compatible with a clinically acceptable blood pressure response. Furthermore, AT-2 receptors might provide endogenous protection to inflammatory, oxidative and apoptotic processes mediated by excessive AT-1 receptors stimulation, although the extent is unknown. As discussed above, serious adverse effects of Ang II occur with the chronic and large dose administration of LJPC-501, but, it is unlikely to happen, as the sponsor notes, if recommended dosing guidelines are followed.

Conclusions

LJPC-501 is intended to raise blood pressure from the hypotensive to normotensive state, and not to induce a hypertensive state. The duration of infusion of LJPC-501 is for a limited period. Furthermore, LJPC-501 has a half-life of approximately 1 minute, so transient hypertension may be promptly mitigated by dose down titration. Identified risks are related to the mechanism of action of LJPC-501, which is manifested as a result of excessive pressor effect on the vasculature, heart and kidney, the target organs of action. The detrimental effect on the target organs are dose- and duration-dependent.

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