CENTER FOR DRUG EVALUATION AND RESEARCH

APPROVAL PACKAGE FOR:

APPLICATION NUMBER

19-386

Pharmacology Review(s)

NDA 19-386: REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA

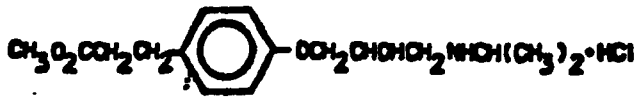
I. SPONSOR: American Critical Care
McGraw Park, Illinois

II. MATERIAL REVIEWED: An original commercial submission dated Oct. 31, 1984 and received by the Center for Drugs and Biologics Nov. 5, 1984. Some of the nonclinical studies were submitted to and reviewed under

III. GENERAL INFORMATION:

A. Chemistry:

1. Trade Name: BREVIBLOC Injection
2. Generic Name: Esmolol HCl
3. Descriptive Name: Methyl 3-(p-Phenoxypropanlamine) proportionate
4. Chemical Name: Methyl 3-[4-[2-hydroxy-3-(isopropylamino) propoxyphenyl]proportionate Hydrochloride
5. Code Numbers: ASL-8052-001; ASL-8052
6. Chemical Structure:



7. Emperical Formula: $C_{16}H_{26}NO_4Cl$
8. Molecular Weight: 331.5
9. Description: White, free flowing , crystalline powder, very soluble in water, freely soluble in alcohol.

B. Pharmacologic Category: Ultra-short acting beta-adrenergic receptor blocking agent.

C. Proposed Indication: Treatment of supraventricular tachycardias and for the management of perioperative tachycardia and hypertension.

D. Dosage Form and Route of Administration: Ampules, 10 ml, containing Brevibloc at 100 mg/ml and 250 mg/ml. Vehicle contains Sodium Acetate, USP; Glacial Acetic Acid, USP; Propylene Glycol, USP; Alcohol, USP; Water for Injecton, USP; Sodium hydroxide, NF and Hydrochloric Acid, NF are used to adjuct pH to 5.0 to 5.3. The vehicle for the 100 mg/ml solution is composed of 10% Propylene Glucol and 10% Alcohol whole that for the 250 mg/ml solution contains 25% Propylene Glycol and 25% Alcohol.

Brevibloc must be diluted to concentrations of no more than 10 mg/ml to avoid vascular irritation and possible thrombophlebitis.

E. Related Documents.

F. Related Compounds:

1. Propranolol HCl (INDERAL)
2. Metoprolol tartrate (LOPRESSOR)
3. Nadolol (CORGARD)
4. Timolol (BLOCADREN)
5. Atenolol (TENORMIN)

IV. PHARMACOLOGY:

A. PHARMACODYNAMICS:

1. Submitted Studies:

PRIMARY THERAPEUTIC ACTIVITY

Characterization of Effects on ASL-052 on Guinea Pig Cardiac and Smooth Muscle in vitro. (ACC, McGaw Park, IL, Research Technical Report, Report No. None; ACC File No. 100-80-00497; Dated Dec., 1980; vol. 1.1, p. 125-141)

Pharmacology of ASL-8052, A novel B-Adrenergic Receptor Antagonist with an Ultrashort Duration of Action. Gorczynski, R.J., Shaffer, J.E. and Lee, R.J., J Cardiovas Pharmacol 5(4):142-151, 1983 (vol. 1.1, p. 142-151)

Beta-Blocking Potency and Pharmacodynamics of ASL-8123. (ACC, McGaw Park, IL, Research Technical Report, Report No. None; ACC File No. 100-84-02436; Dated Oct. 12, 1984; vol. 1.1, p. 152-165)

Duration of Beta-Blocking Action of ASL-8052 in Anesthetized Dogs. (ACC, McGaw Park, IL, Research Technical Report, Report No. None; ACC File No. 100-80-00450; Dated Jan., 1981, vol. 1.1, p. 166-170)

Duration of Beta-Blockade with ASL-8052 in Conscious Dogs. (ACC, McGaw Park, IL, Research Technical Report, Report No. None; ACC File No. 100-80-00454; Dated Jan., 1981; vol. 1.1, p. 171-177)

Cardiovascular Pharmacology of ASL-8052, An Ultra-Short Acting B-Blocker. Murthy, V.S., Hwang, T.F., Zagar, M.E., Vollmer, R.R. and Schmidt, D.H. European J Pharmacol. 94:43-51, 1983 (vol 1.1, p. 178-186)

Beta-Blocking Potency and Duration of Action of Levo-ASL-8052. (ACC, McGaw Park, IL, Research Technical Report, Report No. None; ACC File No. 100-84-00031; Dated Jan. 24, 1984; vol. 1.1, p. 187-204)

Relative Cardiac Inotropic/Chronotropic Blocking Properties of ASL-8052 in Anesthetized Dogs. (ACC, McGaw Park, IL, Research Technical Report, Report No. None; ACC File No. 100-81-00079; Dated Jan., 1981; vol. 1.1, p. 205-221)

Beta-Blocking and Hemodynamic Effects of ASL-8052. Gorczynski, R.J., Murthy, V.S. and Hwang, T.F., Unpublished manuscript from ACC & Mount Sinai Medical Ctr., Milwaukee, WI (ACC File No. 100-84-00477, vol. 1.1, p. 222-259)

Effects of Esmolol, A Short-Acting Beta-Blocker in Enflurane Anesthetized Dogs. Reves, J.G., Kissin, I. and Fournier, S. Anesthesiology 50(3):A58, 1983 (Abstract) (vol. 1.1, p. 260)

Cardioselectivity of ASL-8052 in Anesthetized Dogs. (ACC, McGaw Park, IL, Research Technical Report, Report No. None; ACC File No. 100-80-00452; Dated Jan., 1981; vol. 1.1, p. 261-277)

Effect of ASL-8052 on Incidence of Coronary Occlusion-Induced Ventricular Fibrillation in the Anesthetized Dog. (ACC, McGaw Park, IL, Research Technical Report, Report No. None; ACC File No. 100-82-00216; Not Dated; vol. 1.1, p. 278-283)

Electrophysiological Effects of Intravenously Infused ASL-8052 in Anesthetized Dogs. Murthy, V.S. and Hwang, T.F. Unpublished manuscript from Mount Sinai Medical Ctr., Milwaukee, WI. (ACC File No. 100-84-02411; vol. 1.1, p. 284-295)

SECONDARY THERAPEUTIC ACTIVITY

Hemodynamic and Electrocardiographic Effects of ASL-8052 in the Chronic Unanesthetized Dog. (ACC, McGaw Park, IL, Research Technical Report, Report No. None; ACC File No. 100-82-00003; Dated Dec. 14, 1981; vol. 1.1, p. 296-321)

Spontaneous Heart Rate, Blood Pressure and Beta-Receptor Blocking Actions of ASL-8052 in Anesthetized Dogs. (ACC, McGaw Park, IL, Research Technical Report, Report No. None; ACC File No. 100-80-00451; Dated Jan., 1981; vol. 1.1, p. 322-332)

Cardiovascular Effects of ASL-8052 in Dogs. A Pilot Study. Murthy, V.S. and Hwang, T.F., Unpublished manuscript from Mount Sinai Medical Ctr., Milwaukee, WI. (ACC File No. 100-84-02476; vol. 1.1, p. 333-340)

The Effects of Esmolol (ASL-8052) on the Determinants of Myocardial Oxygen Demand and Supply in Anesthetized Dogs. Murthy, V.S. and Hwang, T.F., Unpublished manuscript from Mount Sinai Medical Ctr., Milwaukee, WI. (ACC File No. 100-84-02412; vol. 1.1, p. 341-351)

First Ultra-Short-Acting Beta-Adrenergic Blocking Agent: Its Effects on Size and Segmental Wall Dynamics of Reperfused Myocardial Infarcts in Dogs. Lang, R., Kloner, R.A. and Braunwald, E. Am. J. Cardiol. 51:1759-1767, 1983. Harvard Medical School. (vol. 1.1, p. 352-360)

Effects of ASL-8052 on Myocardial Infarct Size. (Presumed to be an "In House" study. ACC File No. 100-80-00505; Dated Oct. 16, 1980; vol.1.1, p. 361-367)

The Effects of ASL-8052 on the Electrical Activity of Cardiac Fibers. Witt, A.L., Columbia University. (ACC, McGaw Park, IL, Research Technical Report, Report No. None; ACC File No. 100-81-00632; Not Dated; vol. 1.1, p. 368-396)

Effects of Esmolol on Intraocular Pressure Responses to Intravenous Glucose Infusion in Conscious Rabbits. (ACC, McGaw Park, IL, Research Technical Report, Report No. None; ACC File No. 100-84-01212; Dated June 5, 1984; vol. 1.1, p. 397-403)

MISCELLANEOUS PHARMACOLOGIC ACTIVITY

Direct Cardiovascular Actions of ASL-8052 in Anesthetized, Catecholamine Depleted Dogs. (ACC, McGaw Park, IL, Research Technical Report, Report No. None; ACC File No. 100-80-00453; Dated Jan., 1981; vol. 1.1, p. 404-417)

Summary Report on Local Anesthetic Testing of USABB at Columbia University. (To: R. Stoll, Ph.D., From: R.J. Gorczynski, Ph.D.; ACC File No. 100-81-00498; vol. 1.1, p. 418-419)

Evaluation of ASL-8123 and Propranolol for Local Anesthetic Activity in New Zealand White Rabbits. (ACC, McGaw Park, IL, Research Technical Report, Report No. None; ACC File No. 100-80-00753; Not Dated; vol. 1.1, p. 420-427)

Effects of Esmolol on Bradycardia Responses Elicited by Electrical Stimulation of the Right Vagus Nerve in Dogs. (ACC, McGaw Park, IL, Research Technical Report, Report No. None; ACC File No. 100-84-01211; Dated June 4, 1984; vol. 1.1, p. 428-433)

Importance of Liver Blood Flow in the Duration of Action of ASL-8052. (ACC, McGaw Park, IL. Study No. None; Central File No. 100-81-00761; Dated Nov. 24, 1981; vol. 1.2; p. 297-303)

2. beta-Adrenergic Blocking Activity:

a. In Vitro Studies:

Isolated Guinea Pig Tissues (Table 1)

ASL-8052 is a beta-adrenergic blocking agent that is less potent than propranolol in isolated spontaneously beating atria, paced left atria and tracheal strips (spiral) by factors of about 40X, 140X & 4500X, respectively. Sponsor claims that "ASL-8052 is approximately 80 times more active at cardiac beta-receptors than at tracheal beta-receptors "however, the data presented suggest that ASL-8052 is 24 to 71 times more potent in blocking cardiac (B_1) than tracheal (B_2) receptors. Propranolol was not selective where as metoprolol was 7 times more potent against B_1- than B_2-receptors.

The compound did not have intrinsic sympathomimetic activity in atria from reserpine pretreated animals and showed no depressant effects until concentrations of 10^{-4}M and 10^{-3}M decreased spontaneous rate and force of contraction, respectively. These concentrations were about 15 times that needed to shift the isoproterenol dose-response curve to the right by 100 fold. Similar effects were caused by metoprolol at these concentrations and propranolol decreased both rate and force at $10A^{-5}$M.

Table 1: PA_2 Values-Isoproterenol Challenge

	Left Atrium	Right Atrium	Trachea	Trachea/Atria
ASL-8052	6.54 (1)	7.01 (1)	5.16 (1)	24-71
Metoprolol	7.31 (6)	7.27 (2)	6.45 (19)	6.7-7
Propranolol	8.70 (145)	8.59 (38)	8.81 (4467)	1-0.5

(pA_2 of ASL-8052/pA_2 of tested cpd=relative potency.

b. In Vivo Studies:

Conscious Rabbits: Effect on Blood Pressure and Heart Rate Effects of Isoproterenol: In separate groups of methylatropine-treated rabbits, heart rate and blood pressure responses to increasing doses of isoproterenol (0.031 to 3.1 mcg/kg, i.v.) were determined before and during a 60 min i.v. infusion of either saline or ASL-8052, 1 mg/kg/min. Saline had no effect on resting blood pressure, resting heart rate or responses to isoproterenol. ASL-8052 decreased blood pressure (-4 mmHg), decreased heart rate (-48 bts/min), and decreased heart rate and blood pressure responses to isoproterenol. ASL-8052 inhibited the positive chronotropic action of isoproterenol to a greater extent than the hypotensive action of isoproterenol.

Anesthetized Rabbits: Effects When Infused into The Portal Vein: In methylatropine-pretreated rabbits resting blood pressure, resting heart rate, and responses to isoproterenol (1.0 mcg/kg, i.v.) were determined before and during a 20 min infusion of ASL-8052 at 1.0 mg/kg/min. In one group of animals ASL-8052 was infused into the femoral vein. In a second group ASL-8052 was first infused into the portal vein and then, after recovery, in to the femoral vein.

Inhibition of heart rate responses to isoproterenol, and changes in both blood pressure and heart rate were significantly greater when ASL-8052 was infused into the femoral vein than when infused into the portal vein.

	Heart Rate Change (bts/min)	Blood Pressure Change (mmHg)
Group 1		
Femoral Vein Infusion	-42 ±3	-9 ±1
Group 2		
Portal Vein Infusion	-7 ±1	0
Femoral Vein Infusion	-40 ±3	-9 ±1

Anesthetized Dog:

Beta-Adrenergic Blockage: Dose Range: The beta receptor blocking activity of ASL-8052 was assessed in anesthetized (pentobarbital/barbital) dog by measuring heart rate and blood pressure responses to a standard i.v. dose of isoproterenol (0.5 mcg/kg) before and during the i.v. infusion of the compound at 5 to 160 mcg/kg/min. Heart rate responses to isoproterenol were inhibited by 10% to 75% during infusions of ASL-8052 at 5 to 160 mcg/kg/min (ID_{50}=50±7 mcg/kg/min) where as infusion rates of 80 mcg/kg/min or more were needed to inhibit isoproterenol-induced hypotension (50% inhibition was not obtained). Similar studies were done with metoprolol or propranolol at cumulative doses of 20 to 1280mcg/kg or 10 to 320 mcg/kg, respectively. The ID_{50} against the heart rate effects of isoproterenol was 60±10 mcg/kg and 90± 20 mcg/kg for propranolol and metoprolol, respectively. While all three drugs antagonized heart rate responses to isoproterenol only propranolol caused a dose-related inhibition of the blood pressure effects of isoproterenol that exceeded 30% on the dose-ranges tested.

Effects on baseline: In the anesthetized dog (pentobarbital/barbital) all three drugs caused a dose-related decrease in heart rate which at the highest doses of ASL-8052, propranolol a metoprolol amounted to -22%, -13% and -9%, respectively. Neither ASL-8052 or metoprolol affected arterial blood pressure but propranolol caused a dose-relative increase in diastolic pressure.

Effect on Isoproterenol Dose-Response Curve in Anesthetized (pentobarbital), Ganglion Blocked (hexamethonium, 10 mg/kg, iv) Dogs (5):

Responses to isoproterenol were determined before and 15 min after increasing i.v. infusion rates of ASL-8052 (25, 50 and 100 mcg/kg/min). ASL-8052 caused doses-related parallel shits to the right of the isoproterenol dose-response curves for both HR and CF. There was no significant difference between the pA_2 values or the slopes of the Schild plots between HR and CF.

	In Vivo pA_2 (moles/kg/min)	(mcg/kg/min)	Slope
Heart Rate	7.27 ± 0.09	(17.8)	1.33 ± 0.23
Contractile Force	7.48 ± 0.09	(20.4)	1.24 ± 0.16

pA_2 by Schild Analysis: The -log of the dose (moles/kg/min) of ASL-8025 which requires a 2-fold increase in the dose of isoproterenol to achieve the same response.

Effect on Heart Rate Responses to Sympathetic Nerve Stimulation (right ansa subclavia) in Anesthetized (alpha-chloralose), Vagotomized Dogs:

HR responses to isoproterenol, 0.125 mcg/kg i.v., and ansa stimulation were determined before and during 10 min infusions of ASL-8052 at increasing rates. The isoproterenol and ansa stimulation caused essentially equal increases in HR prior to ASL-8052 administration. ASL-8052 was equally effective in inhibiting heart rate increase caused by sympthetic nerve stimulation and isoproterenol.

	ID50, mcg/kg/min
Isoproterenol	50.0 ± 7.4
Ansa Stimulation	37.0 ± 7.0

c. Duration of beta-Receptor Blocking Actinity in Anesthetized Dogs:

Duration of beta-receptor blockade was determined in anesthetized (pentobarbital/barbital) dogs by measuring the heart rate response to a standard dose (0.5 mcg/kg) of isoproterenol given every 10 minutes before, during and after a 3 hour infusion of ASL-8052 at 50 mcg/kg/min (total=9000 mcg/kg) or propranolol at 1.13 mcg/kg/min (total 203.4 mcg/kg). The onset of beta blockade was faster with ASL-8052 than with propranolol such that steady state blockade with ASL-8052 was achieved in 10-15 minutes. With propranolol, on the other hand, the degree of beta blockade continued to increase for at least 2 hours. The maximum inhibition of isoproterenol was 66% with propranolol and 56% with ASL-8052. At termination of infusion the decrease in the degree of beta-block with ASL-8052 was rapid with 80% recovery in 12±3 min (n=12) and essentially no block at 20 min. With propranolol, in contrast, there was only 22-25% recovery in 1 hour after terminating the infusion.

The duration of beta-receptor blockade with ASL-8052 was similarily determined in conscious ganglion blocked (hexamethonium) dogs by recording heart rate responses to a standard i.v. dose of isoproterenol (0.3 mcg/kg) before, during and after the i.v. infusion of ASL-8052 (20 mcg/kg/min X 3 hours). The time for 80% recovery was 17±3 min and by 20 min following infusion termination there was complete recovery.

Importance of Liver Circulation on the Duration of Action of ASL-8052. Heart rate responses to a standard dose of isoproterenol (0.5 mcg/kg) were determined before, during and after a 3 hour infusion of ASL-8052 (50 mcg/kg/min) in anesthetized (pentobarbital/barbital), vagotomized, ganglion blocked (hexamethonium) mongrel dogs with the hepatic artery occluded. The time for 50% and 80% recovery from beta block on termination of infusion were 9 & 17 min in dogs in which the portal vein was shunted to the femoral veins 5 min prior to terminating the infusion. This time for 50 & 80% recovery are not different than the 8 & 15 min observed in non-shunted dogs. Therefore the liver does not play a significant role in the short duration of action of ASL-8052. Clinical studies are claimed to show that the clearance of ASL-8052 is 20 times greater than liver blood flow following a 1 hour infusion.

d. Cardioselectivity of beta-Receptor Antagonism:

In Vitro: See table 1 under "Isolated Guinea Pig Tissues".

In Vivo: Anesthetized Dog:

Cardio-selectivity: In anesthetized (pentobarbital), vagotomized, ganglion blocked (hexamethonium), dogs heart rate and perfusion pressure (constant flow perfused femoral artery/with aortic ligation) changes to isoproterenol were used to assess the relative activity of ASL-8052, propranolol or metaprolol on cardiac (beta 1) and vascular (beta 2) receptors. ASL-8052 was infused at i.v. doses of 0.01 to 2.56 mg/kg/min or i.a. doses of 0.011 to 0.36 mg/kg/min; propranolol was infused to cumulative doses of 0.01 to 0.32 mg/kg i.v. or to 10.8 mcg/kg i.a.; metaprolol was infused to cumulative doses of 0.02 to 20.48 mg/kg i.v. or 0.045 to 1.44 mg/kg i.a.

Intravenous ASL-8052 had no effect on baseline heart rate (HR) or perfusion pressure (PP) except for a 10 beat/min decrease in the former and 36 mmHg increase in the latter at the fastest infusion rate (2.56 mg/kg/min). High infusion rates of ASL-8052 caused extreme increases in PP. Similar effects were also produced by metoprolol at 20.48 mg/kg whereas propranolol at up to the 0.32 mg/kg cumulative i.v. dose did not change either HR or PP.

Based on regression analysis the doses which caused 50% inhibition of the isoproterenol-induced HR increase and PP decrease were as follows: ASL-8052: 27 mcg/kg/min and 2560 mcg/kg/min; propranolol: 40 mcg/kg and 54 mcg/kg; and metoprolol: 58 mcg/kg and 5360 mcg/kg. These ID_{50} doses result in cardio-selective ratios of 1.37, 95.6 and 115.3 for propranolol, ASL-8052 and metoprolol, respectively. Therefore, ASL-8052 like metoprolol was about 100 times more selective for cardiac than vascular beta receptors while propranolol was non-selective.

When agonist and antagonists were administered i.a. at least 80% inhibition of isoproterenol vasodilation could be achieved and the ID_{50}'s for ASL-8052, propranolol and metoprolol were 160 mcg/kg/min, 1.7 mcg/kg (cumulative) and 470 mcg/kg (cumulative), respectively, i.e. 16, 31 and 11 times less than observed i.v. (Similar slopes suggest a common mechanism).

Relative Inotropic/Chronotropic Blocking Action

Heart rate (HR), blood pressure (BP) and right ventricular contractile force (CF) responses to increasing dose of isoproterenol were determined before and after infusion of ASL-8052 or propranolol in anesthetized (pentobarbital), ganglion blocked (hexamethonium) vagotomized mongrel dogs.

ASL-8052 (25, 50 and 100 mcg/kg/min) caused a dose related inhibition of the positive inotropic and positive chronotropic but not the hypotensive effects of isoproterenol. ASL-8052 tended to block inotropic responses more readily than chronotropic responses (2 fold). Sponsor points out that this inotropic selectivity was small, not seen in the guinea pig isolated preparations, and is probably not clinically significant.

Propranolol (20, 60 or 140 mcg/kg) in experiment performed 1 1/2 yrs previously caused a dose-related inhibition of isoproterenol on CF, HR and BP but there was no selectivity for HR or CF.

Baseline levels CF, HR or BP were not altered by these dose of ASL-8052 or propranolol.

e. Hemodynamic Effects:

Conscious Rabbits: Effects on Blood Pressure (femoral arterial) and Heart Rate. Influence of Methylatropine or Propranolol: ASL-8052 was infused i.v. for 10 min at increasing infusion rates of 0.031 to 3.1 mg/kg/min to separate groups of rabbits which either received no pretreatment or which were pretreated with methylatropine (5 mg/kg, i.v.).

ASL-8052 caused dose-related decreases in heart rate and blood pressure in both untreated and methylatropine treated rabbits. The onset of these effects occurred in 1-2 min, became stable in 6 min and recovered in 20 min. The blood pressure was slightly lower and the her at rate was considerably faster in rabbits treated with methylatropine than in untreated rabbits. Differences between untreated and methylatropine-treated rabbits in terms of minimum effective dose and maximal decreases in heart rate appeared related to differences in resting the levels.

ASL-8052 caused significant and dose-related decreases in blood pressure in both untreated and methylatropine-treated rabbits with a minimum effective dose of 0.31 mg/kg/min in the former and 1.0 mg/kg/min in the latter. The maximum effect was the same in both groups.

ASL-8052 caused significant and dose-related decreases in heart rate at all infusions rates in methylatropine-treated rabbits and at doses of 0.1 mg/kg/min or above in untreated rabbits. Although the magnitude of the heart rate decreases were greater in methylatropine-treated than in untreated rabbits the absolute heart attained were equal (at 3.1 mg/kg/min) or higher (all other doses) in the former than in the latter.

	Resting Level	Minimum Effective Dose[a]	Maximum Effective Dose[a] (Effect)
Blood Pressure			
Untreated	100 ±3 mmHg	0.31	3.1 (-13 ±1 mmHg)
Methyatropine	89 ±2 mmHg	1.0	3.1 (-12 ±2 mmHg)
Heart Rate			
Untreated	244 ±9 bts/min	0.031	3.1 (-32 ±9 bts/min)
Methyatropine	297 ±5 bts/min	0.1	3.1 (-59 ±4 bts/min)

[a] Dose = mg/kg/min.

In a group of propranolol (2 mg/kg, i.v.) pretreated rabbits ASL-8052 infused i.v. at 1.0 mg/kg/min for 60 min had no effect on heart rate but significantly decreased blood pressure by 8 ±1 mmHg. Propranolol alone reduced blood pressure 10 mmHg and reduced heart rate 6 5 bts/min.

Anesthetized (pentobarbital) Rabbits: Effects on Mesenteric Blood flow and Cardiac Output: In one group of animals blood pressure, heart rate and mesenteric blood flow (electromagnetic flow probe) were measured before, during and after a 10 min infusion of ASL-8052 at 1 mg/kg/min. ASL-8052 significantly decreased blood pressure, heart rate and mesenteric arterial resistance but not mesenteric arterial blood flow. At 20 min after terminating the infusion blood pressure and arterial resistance had completely recovered but heart rate was still slightly reduced.

In a group of vagotomized rabbits blood pressure, heart rate and cardiac output (electromagnetic flow probe on ascending aorta) were recorded before and during a 20 min i.v. infusion of ASL-8052 at 1.0 mg/kg/min. Some had received propranolol (2 mg/kg, i.v.). ASL-8052 significantly reduced heart rate, blood pressure, cardiac output and total peripheral resistance. Propranolol decreased cardiac output and heart rate to about the same extent as did ASL-8052. In contrast to ASL-8052, propranolol did not significantly alter blood pressure but did significantly elevate total peripheral resistance. It should be noted that effects of either compound on blood pressure, cardiac out, or total peripheral resistance, though significant, were small. Propranolol prevented the effects of ASL-8052 heart rate and cardiac output but not mean blood pressure or total peripherial resistance.

These experiments indicate that, in the rabbit, ASL-8052 decreased blood pressure by a non-beta-adrenergic receptor mediated vasodilatory mechanism. Under similar conditions propranolol causes vasoconstriction (increases total peripheral resistance).

Conscious Dogs:

Hemodynamic and ECG Effects in Chronically Instrumented Conscious Dogs. Increasing i.v. doses of ASL-8052 (5-160 mcg/kg/min), propranolol (10-320 mcg/kg, cumulative) or metoprolol (20-640 mcg/kg, cumulative0 were administered by 20 minute i.v. infusions to conscious dogs instrumented 3 days previously to record ECG, heart rate (HR) systolic and diastolic arterial blood pressure (SBP & DBP), left ventricular end diastolic pressure (L VEDP), dp/dt, cardiac output (CO, via aortic flow probe), stroke volume (SV), total peripheral resistance (TPR) and pulse pressure (PLP). All dogs (n=6) received each drug on different days and metoprolol was always studied last (n=5).

ASL-8052 at up to 160 mcg/kg/min had no significant effects on HR, SBP, DBP, LVEDP, SV, TPR, QRS complex, T wave or ST-segment. The compound caused a dose-related decrease in dp/dt, max of from -11% at 20 mcg/kg/min to -23% at 160 mcg/kg/min. The highest dose (160 mcg/kg/min) also decreased pulse pressure (-13%) and prolonged the PR-interval (+9%). Pulse pressure changes were due to a nonsignificant decrease in SBP coupled with a nonsignificant increase in DBP. Cardiac output was slightly increased (+12%) at the lowest dose level (5 mcg/kg/min) but returned to control levels at subsequent doses. All effects of ASL-8052 were readily reversible within 20 min of terminating the infusion at the highest rate (160 mcg/kg/min).

Propranolol at up to 320 mcg/kg, (cumulative) had no significant effect on SBP, SV, QRS complex, T wave or the ST-segment. Significant dose-related effects seen with propranolol included a decrease inCO ranging from -9% at 20 mcg/kg to -22% at 360 mcg/kg, a decrease in dp/dt max of from -11% at 40 mcg/kg to -20% at 360 mcg/kg, an increase in TPR from +26% at 20 mcg/kg to +41% at 360 mcg/kg and a prolonged PR-interval of from +13% at 40 mcg/kg to +18% at 360 mcg/kg. Significant increases in DBP (up to +17%) and LVEDP (up to +19%) were seen at 10 to 360 mcg/kg whereas only the high dose (360 mcg/kg) significantly reduced PLP (-17%) and lowered HR (-14%). The above changes did not subside when the infusion was terminated but, in fact, changes in TPR, PLP, DBP and CO continued to increase in magnitude.

Metoprolol, at doses up to 640 mcg/kg did not significantly alter the QRS, T or ST-segment of the ECG, SBP, PLP (tended to decrease by up to -8%), LVEDPT, or SV. The low dose (20 mcg/kg) increased HR by +22% while the high dose (640 mcg/kg) although not statistically significant, decreased HR by 8%. Metoprolol caused a dose-related prolongation of the PR-interval (ranging from +8% at 160 mcg/kg to +13% at 640 mcg/kg), a dose-related decrease in dp/dt (ranging from +8% at 20 mcg/kg to -17% at 640 mcg/kg) and a dose related decrease in CO (that was statistically significant, -19%, at 640 mcg/kg). The high dose (640 mcg/kg) also caused a significant elevation of TPR (+13%) while the low dose (20 mcg/kg) increased DBP (+13%). Like with propranolol, a number of functions continued to change even though the infusion was terminated. These included dp/dt, TPR, and CO.

Beta-Blocking and Hemodynamic Effect in Dogs:

Anesthetized (pentobarbital) Dogs With and With out Ganglionic Blockade:

Arterial blood pressure (BP), heart rate (HR), left ventricular pressure (LVP), left ventricular dp/dt (LV dp/dt), and right ventricular contractile force (CF) (Walton-Brodie strain gauge arch) were recorded before and during the i.v. infusion of saline or ASL-8052. ASL-8052 was infused for 10 minutes and then the infusion rate (dose) was increased. The degree of beta-receptor blockade was assessed during each infusion by recording responses to a standard dose of isoproterenol (0.125 mcg/kg, i.v.). Saline was administered to 6 dogs (3 untreated and 3 pretreated with hexamethonium, 10 mg/kg, i.v.). ASL-8052 was administered to 12 dogs (6 untreated and 6 pretreated with hexamethonium, 10 mg/kg, i.v.).

ASL-8052 (1.58×10^{-8} to 5.01×10^{-7} moles/kg/min or 5.25 mcg/kg/min to 166 mcg/kg/min) produced dose-dependent significant (at each dose) decreases in HR, CF, LV dp/dt and diastolic BP. Systolic BP was unchanged. These effects were not seen in dogs treated with the ganglion blocking agent, hexamethomium.

ASL-8052 also caused dose-related inhibition of the effects of isoproterenol on HR, LV dp/dt, and CF, with nearly complete inhibition at the 166 mcg/kg/min infusion rate. BP effects of isoproterenol were not reduced.

	ID50, mcg/kg/min [a]		
	HR	LV dp/dt	CF
ASL-8052	30.6 ± 4.6	20.3 ± 4.8	23.4 ± 4.6
ASL-8052 + Hexamethonium	16.2 ± 2.6	19.4 ± 2.4	17.3 ± 2.8

[a] Dose causing 50% inhibition of isoproterenol response.

The significantly lower ID_{50} on HR in ganglion blocked dogs appears related to a greater inhibition of the isoproterenol response by the lowest dose of ASL-8052 in these dogs vs. dogs without hexamethonium. The rest of the curve relating HR increase to the standard dose of isoproterenol to ASL-8052-dose looks very similar in the two groups of animals. Thus, the difference may not be meaningful or real. The Sponsor offers no explanation.

Effects on Coronary Blood Flow in Anesthetized (pentobarbital, n = 7) Dogs:

In addition to the hemodynamic functions measured above (excluding CF) blood flow in the circumflex coronary artery was measured using a non-canulating electromagnetic flow probe. Hemodynamic functions and the hyperemic response to 10 sec of coronary occlusion were measured before and at 9 min into a 10 min infusion of ASL-8052 at 31 mcg/kg/min.

ASL-8052 significantly decreased HR, diastolic BP, mean BP, LV dp/dt, the rate-pressure product, and diastolic coronary flow. Systolic BP, systolic and mean coronary blood flow, and mean coronary vascular resistance were not significantly affected. Furthermore, ASL-8052 did not significantly alter the hyperemic response to 10 sec of coronary occlusion nor the time to 50% recovery from the peak hyperemic response.

Anesthetized (pentobarbital) Dog: Effect on Hemodynamics and Myocardial Oxygen Consumption: Femoral arterial blood pressure (BP), heart rate (HR), left ventricular pressure (LVP), left ventricular dp/dt (LV dp/dt), circumflex coronary arterial blood flow (CBF), and myocardial oxygen consumption (MVO) were determined before, during isoproterenol infusion (0.1 mcg/kg/min X 10 min), during ASL-8052 infusion (310 mcg/kg/min X 15 min), during the combined infusion of ASL-8052 and isoproterenol, and again 30 min after all infusions were terminated. MVO was determined by the difference in oxygen content between blood samples taken from the left ventricle (arterial) and coronary sinus (venous).

Isoproterenol significantly increased systolic BP, HR, LV dp/dt, CBF, and MVO; decreased diastolic BP and coronary vascular resistance, and did not alter oxygen extraction.

ASL-8052 significantly decreased HR, LV dp/dt, CBF and MVC; increased coronary vascular resistance, and did not affect systolic BP, diastolic BP or oxygen extraction. All effects of ASL-8052 were slight consisting of changes of 20% of less.

During the infusion of ASL-8052 all effects of isoproterenol were markedly reduced or abolished. Thirty (30) min after terminating the infusion of ASL-8052 responses to isoproterenol returned to that seen prior to ASL-8052 infusion.

Hemodynamic Effects in Enflurane Anesthetized Dogs (n = 5):

Systolic blood pressure (SBP), diastolic blood pressure (DBP), heart rate (HR), right atrial pressure (RAP), left atrial pressure (LAP), Cardiac output (CO) (thermodilution), and left ventricular contractile force (LVCF) (strain gauge arch) before and during 20 minute infusions of ASL-8052 at 100, 300 and 1000 mcg/kg/min. The degree of beta blockade was assessed by administration of isoproterenol at 1.0 mcg/kg. i.v.

HR responses to isoproterenol were reduced at 100 mcg/kg/min and abolished at 300 mcg/kg/min of ASL-8052.

		% Change of Hemodynamic Parameter		
	Control	ASL-8052, mcg/kg/min		
	Level	100	300	1000
HR (bts/min)	118 ±27.8	-9.3	-14.4*	-19.5*
CF (% Control)	0	-21.0*	-37.0*	-52.0*
SBP (mmHg)	145 ±68.4	+9.0	-1.4*	-26.9*
DBP (mmHg)	84 ±34.8	-4.8	-17.9*	-44.0*
LAP (mmHg)	1.4 ±0.55	+42.9	+28.6	+100.0*
CO (l/min)	2.7 ±0.85	-11.1	-7.4	-22.2

* P = 0.05 of less. Based on absolute change from control level except CF which was based on % of control.

f. Intrinsic Sympathomimetic Activity (ISA) and/or Myocardial Depressant Activity: See "beta-Adrenergic Blocking Activity: In Vitro studies: Isolated guinea pig tissues."

The following two summaries refer to studies submitted in the Original IND submission and in the NDA, respectively. It appears that there was only one study but this was not clear from either submission since each report contained slightly different sets of data. Therefore, both summaries are presented below.

Cardiac Intrinsic Sympathomimetic and/or Depressant Activity. Heart rate, right ventricular contractile force (Walton Brodie strain gage), left ventricular pressure and dp/dt were recorded in paced (right atrium) and unpaced anesthetized (pentobarbital), reserpinized (0.1 mg/kg ip X 7 days), vagotomized dogs before and during the administration of increasing doses of ASL-8052 (0.33-3.8 mg/kg/ml), propranolol (2-41.56 mg/kg) or metoprolol (2.0-41.56 mg/kg). Doses of propranolol and metoprolol are cumulative. Dose of each antagonist was increased until contractile force (CF) was reduced by at least 70%.

	% change from control		
	RVCF	HR	BP
ASL-8052, 2.53 mg/kg/min	-46	-9	-45
Propranolol, 26.38 mg/kg	-51	-29	-56
Metoprolol, 16.25 mg/kg	-47	-18	-24

Sponsor did not present data on dp/dt. At the highest infusion rate of ASL-8052 (3.8 mg/kg/min), BP decreased to 31 mmHg, HR decreased to 118 bts/min and CF decreased to 34% of control. All functions rapidly returned to control levels within 10 min of terminating the infusion in 3/6 dogs; in the remaining 3 dogs CV depression was too great for recovery.

None of the compound had intrinsic sympathomimetic activity. It was frequently not possible to pace the hearts following administration of propranolol, therefore, only unpaced data are presented.

All three antagonists caused a dose-related decrease in CF and right atrial pacing made little or no difference. The minimum effective dose for ASL-8052, propranolol and metoprolol was 0.75 mg/kg/min, 5 mg/kg (cumulative) and 5 mg/kg (cumulative), respectively. The doses causing a 50% reduction in CF (DD_{50}) were: ASL-8052: 1.65 mg/kg/min, unpaced & 1.66 mg/kg/min, paced; propranolol: 26 mg/kg, unpaced; metoprolol: 11.6 mg/kg, unpaced & 18.5 mg/kg, paced. The beta-receptor blocking dose (ID_{50}, isoproterenol) for ASL-8052, propranolol and metoprolol were 0.05 mg/kg/min, 0.06 mg/kg and 0.09 mg/kg, respectively. Thus the relative safety ratios (DD_{50}/ID_{50}) were 33, 433 and 129-306 for ASL-8052, propranolol and metoprolol.

The sponsor states that "the depression in heart rate associated with a 50% depression of RVCF was smaller with ASL-8052 (-12±3) then with either propranolol (-35±7) or metoprolol (-26±3)." Since these values are not labeled it is unclear whether they refer to beats/min or a %. From the table below it is clear that at doses that reduce RVCF by about equal amounts, all three antagonist lowered blood pressure and reduced heart rate.

Intrinsic Sympathomimetic Activity (ISA) and Cardiac Depression vs. Metoprolol in Anesthetized, Reserpinized, Vagotomized Dogs:

Arterial BP, HR, LV dp/dt and right ventricular CF (Walton-Brodie strain gauge arch) measured with and without right atrial pacing before and during 10 min i.v. infusions of increasing doses of ASL-8052 (0.3 to 3.8 mg/kg/min) (n = 6) of metoprolol (dose range not stated) (n =6). Note that the lowest dose of of ASL-8052 is at least 10 times larger than the in vivo pA_2.

While un-paced, ASL-8052 had no ISA and doses of 0.75 or 1.13 mg/kg/min and above cause dose-related reductions of BP, HR, CF, LV dp/dt and increased LV end-diastolic pressure. Similar results were said to have occurred with metoprolol but these data were not presented. Sponsor states that the slope of the decrease in CF vs. log of the infusion rate was greater for ASL-8052 than for metoprolol. At doses that caused equivalent decreases in either CF or dp/dt both ASL-8052 and metoprolol caused similar decreases in BP but ASL-8052 caused significantly less slowing of heart rate.

Right atrial pacing did not alter the the doses causing cardiac depression.

	DD_{50} Dose for 50% Depression		Decreases at DD 50 for CF & dp/dt HR,bts/min		BP, mmHg	
	CF	dp/dt	CF	dp/dt	CF	dp/dt
ASL-8052 (mg/kg/min)						
Unpaced	1.65 ±0.21	1.31 ±0.16	-11	-7	-53	-26
Paced	1.66 ±0.14	1.52 ±0.13	-	-	-54	-38
Metoprolol (mg/kg, cumulative)						
Unpaced	18.3 ±3.9	11.6 ±1.0	-26	-31	-35	-21
Paced	17.4 ±1.4	18.5 ±1.5	-	-	-33	-35

The highest doses of both metoprolol and ASL-8052 caused marked decreases in BP, CF and dp/dt, and marked increases in LV end-diastolic pressure. When the infusion of metoprolol was terminated there was no recovery in 4/4 dogs whereas termination of the ASL-8052 resulted in complete recovery in 10 min (recovery started in 1 min) in 3/4 dogs (1 dog died)

g. Local Anesthetic Activity:

Frog Sciatic Nerve

Neither ASL-8052 at up to 0.1M nor timolol at 0.01M reduced the amplitude of the extracellularly recorded action potential. Propranolol at 0.01M, however, reduced the amplitude by 99%. Thus ASL-8052 like timolol, but unlike propranolol, did not have local anesthetic properties.

Corneal and Infiltration Anesthesia - Rabbit:

ASL-8052 was dissolved in 0.9% saline and pH adjusted to 5 with HCl. Dilutions of Xylocaine (lidocaine 4%) were also made with saline. Timoptic (timolol) was tested at the 0.5% commercial preparation. The pH of the xylocaine, timolol and saline were 7.0, 7.0 and 5.5, respectively.

Corneal Anesthesia: ASL-8052 at 1%, 3% and 10%, lidocaine at 1% and 4%, and Timoptic, 0.5% were evaluated in each of 4 rabbits for corneal anesthesia. The cornea was stimulated with the blunt edge of polyethylene tubing (11 times at 60/min) both before and after the instillation of 0.1 ml of each solution into one eye (eye lids healed closed for 30 sec) of separate rabbits. For control, saline was applied to the contralateral eye. ASL-8052 at 1% caused no corneal anesthesia. However, concentrations of 3% produced slight transient anesthesia and ant 10% only partial (incomplete, i.e., blinking to some of the 11 stimuli) anesthesia which peaked at 10 min and lasted less than 30 min. This effect was essentially the same as produced by 4% lidocaine, a relatively poor corneal anesthetic. Timolol (0.5%) did not cause corneal anesthesia.

Infiltration Anesthesia: ASL-8052 at 0.03%, 0.1%, 0.3%, 1%, 3% and 10%, Lidocaine at 0.4%, 1% and 4%, and Timoptic, 0.5%, were evaluated in each of 4 rabbits for infiltration anesthesia. Rabbits received 0.5 ml, s.c. of each solution on the shaved back. Anesthesia was evaluated, by stimulation (11 times at 60/min) with a 27 gauge hypodermic needle, bothe before and up to 4 hours after compound administration. ASL-89052 at 0.03% and 0.1% produced no changes. The 0.3% solution produced partial anesthesia and slight edema lasting 150 min. The 1%, 3% and 10% solutions of ASL-8052 caused complete anesthesia of immediate onset and lasting longer than 4 hrs, which was accompanied by the slow development of marked edema. It is unlikely that the edema was due to the low pH since it did not occur with saline (pH 5.5) and was concentration-dependent. Edema was not seen with lidocaine or Timoptic. Lidocaine at 0.4% cause only partial anesthesia of short duration but at 1% and 4% caused complete anesthesia with an onset of 30 min and a duration of less than 2 hrs. The magnitude and duration of anesthesia produced by ASL-8062 at 1% and lidocaine at 4% were similar. Timoptic did noit cause infiltration anesthesia.

Thus, ASL-8052 was an effective infiltration anesthetic at concentration of 0.3% or greater and, as such, was more potent than lidocaine. In addition, the infiltration anesthesia caused by ASL-8052 but not lidocaine was associated with edema which at concentrations above 1% was marked. ASL-8052 was also an effective corneal anesthetic at concentrations of 3% and above but in this case was less potent than lidocaine. Timoptic at 0.5% was ineffective as a corneal or infiltration anesthetic.

h. Electrophysiologic Effects:

Isolated Cardiac Tissues:

Rabbit SA Node: ASL-8052 at low concentrations (10^{-7}M to 10^{-4}M) tended to increase automaticity by 6-15%, where as, 10^{-3}M decreased automaticity by 36%.

Concentrations of 10^{-4}M and above decreased diastolic resting potential, Vmax, action potential amplitude and overshoot.

At concentrations of 10^{-6}M to 10^{-4}M, ASL-8052 caused a concentration-dependant, competitive inhibition of the positive chronotropic action of NE. This effect was readily reversed by washing and suggests a weak interaction with B receptors which may play a role in the short in vivo action of the compound.

Rabbit AV node: ASL-8052 at concentrations of 10^{-6}M to 10^{-5}M blocked the positive chronotropic action of NE and the NE-induced acceleration of AV nodal conduction.

ASL-8052 at 10^{-7}M caused a slight acceleration of AV nodal conduction and concentrations up to 10^{-5}M had no effects on AV nodal conduction or effective refractory period (ERP). These functions were slowed and prolonged, respectively, at concentrations of $6X10^{-5}$M or 10^{-4}M. ASL-8052 caused AV block at 10^{-3}M.

All effects of ASL-8052 were readily reversed by washing.

Canine Purkinje Fibers

ASL-8052 at 10^{-7}M to 10^{-5}M blocked NE-induced automaticity and NE-induced slow response in K^+depolarized fibers (without altering diastolic potential).

Resting diastolic potential was slightly increased (hyperpolarization) by ASL-8052 at 10^{-7}M to 10^{-5}M but reduced (depolarized) at 10^{-3}M.

Action potential amplitude, V max, and action potential duration (APD) decreased at 10^{-4}M but unaffected at 10^{-5}M or below. The fibers were inexcitable at 10^{-3}M. At 10^{-4}M the APD to 50% repolarization was shorted (by shifting the plateau to a more negative value). Although sponsor states that the APD at 100% repolarization was prolonged data presented do not clearly indicate that the ADP 100 was prolonged by ASL-8052 significantly beyond the duration seen in controls or after a low concentration (10^{-8}M) of compound. The ERP was not affected by ASL-8052 at up to 10^{-4}M.

Thus beta-receptor blockade with ASL-8052 could be demonstrated at concentrations of 10^{-7}M to 10^{-5}M but direct effects of the compound occurred at concentration of 10^{-4}M or greater.

In vivo: Anesthetized (pentobarbital) Dog: Arterial blood pressure, surface ECG and atrial, ventricular and HIS electrograms were recorded before, during and 30 after i.v. infusion of ASL-8052 at 310 mcg/kg/min for 30 min. Recording were made during spontaneous sinus rhythm as well as during right atrial pacing (incrementally or at 300/min with and without timed extra stimuli).

In unpaced dogs, ASL-8052 prolonged The P-P interval (slowed HR) and prolonged the PR interval but did not significantly affect blood pressure, QRS duration, AH interval or HV interval.

During atrial pacing at 300/min ASL-8052 prolonged the simus node recovery time, prolonged the SH (S = stimulus) and AH intervals but did not affect the HV interval.

ASL-8052 prolonged the relative and functional refractory periods of the AV node, and prolonged the Wenckebach cycle length (cycle length during incremental pacing when an atrial depolarization is not followed by a HIS depolarization).

The recorded functions returned to or near control levels 30 min after terminating the infusion. Since stability of the preparation was not tested failure to return to control levels can not be interpreted as continued drug action.

1. Antiarrhythmic Activity:

Coronary Occlusion Arrhythmias - Anesthetized (pentobarbital) Dog: In separate groups of 6 dogs with heart rate equal to or greater than 155 bts/min saline or ASL-8052 (0.15 mg/kg/min) was infused i.v. for 20 min prior to LAD occlusion and continued until ventricular fibrillation (VF) or for 30 min.

	Saline	ASL-8052
Heart Rate, bts/min:		
Before infusion, bts/min	187 ±11	186 ±10
After infusion, before Occlusion	187 ±10	151 ±9
Mean Blood Pressure, mmHg:		
Before infusion, bts/min	143 ±6	142 ±3
After infusion, before Occlusion	144 ±7	140 ±4
Ventricular Tachycardia	6/6	4/6
Ventricular Fibrillation	4/6	0/6

Summary: In anesthetized dogs infusion of ASL-8052 at 150 mcg/kg/min for 20 min did not significantly affect blood pressure but did reduce heart rate. When the LAD was occluded the compound reduced the incidence of ventricular tachycardia and prevented ventricular fibrillation.

Effect on Bradycardia Induced by Electrical Stimulation of the Right Vagus Nerve - Anesthetized (pentobarbital) Dog: In separate groups of propranolol (1 mg/kg, i.v.) treated dogs, heart rate and blood pressure responses to electrical stimulation of the peripheral end of the cut right vagus nerve were determined before and during the i.v. infusion of saline for 60 min or ASL-8052 for 60 min (at 100 and 300 mcg/kg/min each for 30 min). The bradycardia induced by vagal stimulation was submaximal reducing heart rate by 40 to 60 beats/min but did not produce A-V dissociation. Responses to vagal stimulation were not altered by either saline of ASL-8052.

Since these doses of ASL-8052 are 2 and 6 times larger than the ID_{50} for a standard dose of isoproterenol in anesthetized dogs the compound should not modify vagally induced cardiac slowing within the beta-receptor blocking range of 5 to 300 mcg/kg/min.

j. Myocardial Infarct Size - Anesthetized Dog:

Blood pressure (carotid artery) and ECG were recorded in anesthetized (pentobarbital), open chest mongrel dogs prepared with a critical stenosis (no reduction in blood flow but abolition of reactive hyperemia) of the left circumflex artery both before and after a 60 minute period of total occlusion of the circumflex artery followed by reperfusion (with the critical stenosis). Saline or ASL-8052 (50 mcg/kg/min) was infused for 20 minutes prior to total occlusion, during occlusion and for 4 hours post reperfusion at which time the chest was closed. One hour later (5 hrs post reperfusion) the dog was returned to its cage. The following day (24 hrs. post surgery) the dog was sacrificed and the heart examined for infarct size using nitroblueterazolium stained slices. One saline and none of the ASL-treated dogs fibrillated (during the 60 min. total occlusion). There were no significant differences between saline and ASL-8052 treated dogs in terms of HR or BP measured just prior to occlusion, 20 to 60 minutes into occlusion or for up to 5 hours after reperfusion. However, infarct size was significantly less in the ASL-8052 treated dogs (7.5% of LV) than in the saline-treated dogs (24% of LV).

Anesthetized (thiamylal) Dog: Effect on Size and Segmental Wall Dynamics of Reperfused Myocardial Infarcts: Arterial blood pressure (BP), left ventricular systolic pressure (LVSP), left ventricular end-diastolic pressure (LVEDP), left ventricular dp/dt (LV dp/dt), heart rate (HR), end-systolic segmental length (ESL) and end-diastolic segmental length (EDL) were continuously recorded before, during occlusion of the left anterior descending coronary artery (LAD) for 3 hrs, and for 3 hrs of reperfusion. All dogs received lidocaine, 1.5 mg/kg, i.v. immediately after occlusion of the LAD. Starting 15 min after occlusion of the LAD, one group of dogs were infused with saline and one group with ASL-8052 (100 to 150 mcg/kg/min, sufficient to reduce HR 20%). The infusions were continued to the end of the experiment.

Authors note that in 2 dogs the positive chronotropic effect of i.v. isoproterenol, 0.5 mcg/kg, was restored in 16 minutes after discontinuing the infusion of ASL-8052 at the above rate for 60 min. No comment is made regarding the degree of beta-receptor blockade during the infusion. Purpose seems to demonstrate the short activity of ASL-8052.

Four (4) dogs fibrillated within 15 min of LAD occlusion, i.e., before infusion of saline or ASL-8052 was started.

On reperfusion saline-treated dogs (8/10) but not ASL-8052-treated dogs, had tachyarrhythmias.

Hemodynamic effects seen in both saline- and ASL8052-treated dogs during 3 hours of LAD occlusion consisted of decreases in LVSP, SBP, DBP, and LVdp/dt. The Decrease of LVdp/dt was greater at 60 and 120 but not 180 min in the ASL-8052 group than the saline group. In addition, HR decreased in the ASL-8052 group but not in the saline group. On reperfusion the difference in HR between the two groups became larger since HR increased in the saline but not the ASL-8052 group.

Segmental Function: Non-ischemic areas: In both saline and ASL-8052 treated dogs the EDL increased slightly but equally during occlusion and generally returned toward control levels on reperfusion. The %SS did not change in either group either during occlusion or on reperfusion (segmental shortening). Border Zone: EDL increased equally in both treatment groups during occlusion and returned toward control levels on reperfusion. The %SS decreased to negative values in both groups during occlusion, i.e. there was no segmental shortening but actually passive systolic stretch (systolic bulging). On reperfusion this condition worsened in the saline- but not the ASL-8052-treated dogs.

Local Blood Flow: At 10 minutes of occlusion, blood flow in the central ischemic zone was 10-15% of that in the normal zone with a slightly greater reduction in endocardial than epicardial flow. On reperfusion flow tended to increase in the epicardium at the expense of the endocardium. There were no significant differences between saline and ASL-8052 treated dogs.

Infarct Size: The infarct size, expressed as a % of area at risk, was significantly smaller in ASL-8052-treated dogs (48 $\pm$ 7%) than in saline-treated dogs (73 $\pm$ 6%).

*Summary: In anesthetized dogs ASL-8052 reduced heart rate before and during coronary artery occlusion, prevented heart rate increases and tachyarrhythmias on reperfusion, prevented the worsening of systolic bulging on reperfusion, and significantly reduced the infarct size. The compound did not depress the nonischemic zone, alter flow during occlusion or affect the area at risk. The beneficial effect was probably due to a decrease in the oxygen requirement due primarily to a reduction in heart rate and prevention of reperfusion tachycardia. As indicated by the Authors, these data suggest that the agent may be potentially useful in the early treatment of patient with acute MI about to undergo early revascularization, either thrombolytic or surgical.

k. Effect on Intraocular Pressure (IOP) - Conscious Rabbits:

In conscious New Zealand albino Rabbits the i.v. administration (over 15-20 sec) of 5% glucose increases IOP with a peak at 5-10 min and recovery at 30 min. In separate groups of rabbits either saline (50 mcl) or 3% ASL-8052 (50 mcl or 150 mcg) were instilled into the right eye either 1 hour or 4 hour before challenge with 5% glucose. The left eye was an untreated control.

ASL-8052 reduced (by about 50%) the glucose-induced increase in IOP in both the treated and contralateral eye at 1 but not 4 hrs. Time intervals earlier than 1 hour after treatment were not tested. Since ASL-8052 was instilled into only one eye but both eyes were affected it is clear the ASL-8052 was absorbed systemically. It is not clear that ASL-8052 affected the treated eye by local absorption or only after systemic absorption. Since the compound is rapidly metabolized by esterases in the blood, the beneficial effect seen 1 hour after administration in the contralateral eye suggests that conjunctival instillation delivers drug systemically much like an i.v. infusion.

l. Levo-ASL-8052 vs dl-ASL-8052:

Beta Blocking Potency and Duration of Action: In vitro, Isolated Guinea Pig Tissues: In the isolated spontaneously beating right atrium l-ASL-8052 was slightly less potent than the racemate, dl-ASL-8053, as a beta adrenergic receptor antagonist. In atrial preparation from catecholamine-depleted (reserpine pretreated) guinea pigs, l-ASL-8052 failed to affect spontaneous rate (right atria) or developed force (stimulated left atria) at concentrations of 1×10^{-8} to 3-9.95 mcg/ml $\times 10^{-5}$M. Higher concentrations (3×10^{-4}M and above) of 99.5 mc/ml l-ASL-8052 caused concentration-related decreases in both spontaneous right atrial rate and force of left atrial contractions with 50% Depression Concentrations (DC_{50}) of 1.74×10^{-4}M and 8.7×10^{-4}M, respectively; dl-ASL-8052 was about 2X more potent in depressing spontaneous rate ($DC_{50} = 9.6 \times 10^{-5}$M) but about equipotent in depressing force of contraction ($DC_{50} = 9.8 \times 10^{-4}$M).

In vivo, Anesthetized, Vagotomized Dog.

Hemodynamic and beta-Blocking Activity: Blood pressure (BP) and heart rate (HR) responses to isoproterenol (0.5 mcg/kg, i.v.) were measured before, during and following the i.v. infusion of l- or dl-ASL-8052 for 10 minutes at increasing doses.

Both l- and dl-ASL-8052 caused dose-related decreases in HR but neither altered BP.

Both compounds inhibited the positive chronotropic effect of the standard dose of isoproterenol. The l- isomer was about 2X more potent than the racemate (dl-ASL-8052) as a beta-receptor antagonist. Neither compound inhibited the hypotensive effect of isoproterenol even at up to 113.9 mcg/kg/min.

	ID_{50} (mcg/kg/min)
l-ASL-8052	29.3 ± 1.7
dl-ASL-8052	56.1 ± 2

Duration of Action: Infusion of l-ASL-8052 at 30 mcg/kg/min for 3 hours decreased spontaneous HR by about 10 bts/min, and inhibited the positive chronotropic action of isoproterenol by 62 ± 3%. The onset of beta-receptor antagonism was rapid, reached a steady stat in 10-20 minutes and disappearance rapidly when the infusion was terminated (the HR response to isoproterenol recovered by 50% in 8 ± 2 min and by 80% in 24 ± 4 min).

The hypotensive effect of isoproterenol was not altered by l-ASL-8052.

Summary of previous in vivo studies with the racemate, dl-ASL-8052:

	% Inhibition of HR response to Isoproterenol	Recovery, min 50%	Recovery, min 80%
50 mcg/kg/min	52	-	-
100 mcg/kg/min	68	7	17

m. Primary Acid Metabolite, ASL-8123:

In vitro beta-Blocking Activity: ASL-8123, the primary acid metabolite of ASL-8052, was also tested on isolated spontaneously beating guinea pig right atria and, with a pA_2 of 4.45 (12.5 mcg/ml), was about 350X less potent than the parent compound as a beta blocking agent. Two batches of the metabolite were tested in 4 experiments each and while both batches were equipotent as beta- blockers only one batch had a slight positive chronotropic effect (+19 bts/min) at 1×10^{-4}M . The other batch did not significantly affect the spontaneous rate.

In vivo: Anesthetized Dog: ASL-8123 was infused i.v. at 0.1, 0.2, 0.4, 0.8, 1.6, 3.2 and 6.4 mg/kg/min for 20 min each (total: 2, 6, 14, 30, 62, 126, and 254 mg/kg). In 5/6 dogs doses of 0.8 to 6.4 mg/kg/min caused dose-related decrease in heart rate of from -17 to -31 bts/min. There was a slight increase in heart rate of 10-11 bts/min in 1/6 dogs. The two highest infusion rates decreased blood pressure 11 21 mmHg, respectively.

The positive chronotropic effect of isoproterenol at 0.5 mcg/kg. i.v. was inhibited at all doses in a dose-related manner ranging from 17% inhibition at the lowest dose to 87% inhibition at the highest dose. Only the two highest doses inhibited the hypotensive effect of isoproterenol (-29% and -41%, respectively).

Venous blood levels of this metabolite, ASL-8123, increased linearly with rate of infusion and correlated with the degree of inhibition of the isoproterenol-heart rate effect: slope = 48.5 ±7.4 % inhibition/mcg/ml. The concentration of ASL-8123 corresponding to 50% inhibition averaged 59.0 ±7.4 mcg/ml. From previous studies the corresponding level of the parent compound, ASL-8052 of 50% inhibition was 0.185 ±0.04 mcg/ml.

Summary: ASL-8123, the primary acid metabolite of ASL-8052 is a competitive, cardio-selective beta-receptor antagonist which in vitro and in vivo is less potent that ASL-8052 by factors of 350 fold and 320 fold, respectively.

Local Anesthetic Activity: New Zealand White Rabbit: Surface (Corneal): Instillation of 0.1 ml of ASL-8123 at 1%, 3% or 10% (1, 3, 10 mg total) did not cause corneal anesthesia. Similarily administered propranolol caused anesthesia at all concentrations tested (0.1 % and above) with complete anesthesia at 1%. Infiltration: ASL-8123 administered s.c. at 0.5 ml of 1%, 3% and 10% (5, 15 & 50 mg, respectively) caused slight anesthesia and edema. Concentrations of 0.1% and 0.3 % were inactive. Propranolol, on the other hand, caused marked anesthesia at concentrations of 0.1% to 10%.

ASL-8123 was inactive as a topical anesthetic and had only slight activity by infiltration. Previous studies indicated the ASL-8052, the parent compound, was 1/600 as active as propranolol in causing corneal anesthesia but about equal to propranolol in causing infiltration anesthesia. Thus, the primary metabolite of ASL-8052 is less active than the parent compound as both a surface and infiltration anesthetic.

B. PHARMACOKINETICS:

1. Submitted Studies:

Protein Binding of Esmolol and its Major Metabolite, ASL-8123. (ACC, McGaw Park,IL. Study No. CPDM-8052(C)-83-07, Dated March 20, 1984; vol. 1.2, p 1-15).

Biochemical Characterization of Blood Esmolol Esterase. (ACC, McGaw Park,IL. Central File MNo. 100-84-00389, Dated Oct., 1983; vol. 1.2, p 16-59).

In Vitro Metabolism of Esmolol by Female Human Blood. (ACC, McGaw Park,IL. Study No. CPDM-8052-84-02; Central File No. 100-84-01216, Dated June, 1984; vol. 1.2, p 60-66).

Effect of Esmolol on the Hydrolysis of Benzoylcholine by Human Blood and Plasma. (ACC, McGaw Park,IL. Study No. CPDM-8052-84-03; Central File No. 100-84-01215, Dated June, 1984; vol. 1.2, p 67-76).

Lack of Interaction Between Esmolol and Succinylcholine in Anethetized Dogs. (ACC, McGaw Park,IL. Study No. None; Central File No. 100-83-01742, Dated Nov. 21, 1983; vol. 1.2, p 77-94).

Mass Balance Study of 14C-8052 in Rats. (ACC, McGaw Park,IL. Study No. 8052-DM-81-01; Central File No. 100-81-00768, Dated 1981; vol. 1.2, p 95-148).

Distribution of Esmolol and its Acid Metabolite in Blood and Various Organs of Rat. (ACC, McGaw Park,IL. Study No. CPDM-8052-84-01; Central File No. 100-84-01254, Dated June, 1984; vol. 1.2, p 149-238).

Pharmacokinetics of Esmolol in Dogs. (ACC, McGaw Park,IL. Study No. CPDM-8052-84-05; Central File No. None Indicated, Dated August, 1984; vol. 1.2, p 239-274).

Pharmacodynamics of ASL-8052 in Anesthetized Dogs. (ACC, McGaw Park,IL. Study No. Not Indicated; Central File No. 100-82-00153, Dated Dec., 1981; vol. 1.2, p 275-296).

Importance of Liver Blood Flow in the Duration of Action of ASL-8052. (ACC, McGaw Park, IL. Study No. None; Central File No. 100-81-00761; Dated Nov. 24, 1981; vol. 1.2; p. 297-303)

In Vitro Hydrolysis of ASL-8052 and ACC-9089 in Rabbit Whole Blood and Ocular Tissue. (ACC, McGaw Park,IL. Study No. Not Indicated; Central File No. 100-82-00611, Dated May, 1982 vol. 1.2, p 304-320).

Ocular Tissue Distribution of 14C-ASL-8052 After Ocular Administration. (ACC, McGaw Park,IL. Study No. CPDM-GLAUMOMA-82-03; Central File No. 100-82-00812, Dated July, 1982 vol. 1.2, p 321-348).

Systemic Absorption of 14C-ASL-8052 After Ocular Administration - A Pilot Study. (ACC, McGaw Park,IL. Study No. CPDM-GLAUMOMA-82-02; Central File No. 100-82-00597; Dated May, 1982 vol. 1.2, p 349-373).

Ocular Tissue Distribution and Systemic Absorption of 14C-ASL-8052 in Mongrel Dogs. (ACC, McGaw Park,IL. Study No. CPDM-GLAUMOMA-82-04; Central File No. 100-82-01146; Dated Oct., 1982, vol. 1.2, p 374-408).

2. METABOLISM:

a. Role of the Liver: In anesthetized dogs, shunting portal vein blood to the femoral veins (i.e., by passing the liver) 5 min prior to terminating a 3 hour infusion of ASL-8052 at 50 mcg/kg/min did not prolong the recovery from beta-adrenergic receptor blockade. This indicates that the liver does not play a significant role in the short duration of action of ASL-8052. Clinical studies are said to indicate that the clearance of ASL-8052 is 20 times greater than liver blood flow following a 1 hour infusion.

b. In vitro Hydrolysis in Rabbit Whole Blood and Ocular Tissue:

Rabbits were screened for fast and slow esterase activity toward ACC-9089.

Tissue	T1/2 (min)
Blood	8.1
Aqueous Humor	none
Cornea	23.1
Iris-Ciliary Body	4.1
Lens	62.4
Sclera	42.1
Vitreous humor	none

ASL-8052 esterase activity was present (in decreasing order) in the iris-ciliary body, blood, cornea, sclera and lens but not in aqueous or vitreous humor.

Sponsor does not indicate the nature of ACC-9089 but data presented indicate that it is hydrolyzed by different esterases than is ASL-8052 and that tissues of the eye like blood have fast and slow esterase activity.

NOTE: Sponsor makes no comment as to whether the rate of hydrolysis of ASL-8052 varies with fast and slow esterase activity identified by ACC-9089.

c. Biochemical Characterization of Blood Esmolol Esterase:

Effect of Concentration on the In Vitro Half-Life of Esmolol in Dog Blood

Esmolol (mcg/ml)	Half-life (min)
0.25	14.5
1.0	10.9
2.5	7.7
25.0	10.1

Thus, the in vitro half-life of esmolol was independent of substrate concentrations suggesting that hydrolysis of esmolol by dog blood follows 1st order kinetics.

Localization of Esmolol Esterase Activity in Dog Blood (Esmolol, 25 mcg/ml)

Fraction	% Whole Blood Activity [1]
Plasma	0.0
Unwashed RBC	52.0 ±4.3
Unwashed RBC + 100% Plasma[2]	97.0 ±2.4
Washed RBC + Saline	16.0 ±3.7
+ Buffer	41.5 ±0.1
+ 25% Plasma	53.4
+ 50% Plasma	65.4
+ 100% Plasma	72.3 ±10.4
+ Buffer + 0.1 M Mg++	39.7 ±2.5
RBC Membrane + Plasma	0.0
RBC Lysate + Saline	23.0
+ Plasma	63.0

[1] The in vivo half-life in dog blood was 15.1 ±3.33 min. Values represent mean ± SD.

[2] The amount of plasma added as a % of amount in blood.

Thus, esmolol esterase activity was not in plasma or erythrocyte membranes but in the erythrocyte cytosol (lysate). Although plasma had no activity alone or when combined with erythrocyte membranes it did enhance the activity or erythrocytes (unwashed and washed) and erythrocyte cytosol. The buffering capacity of plasma may play a role in its ability to enhance the esterase activity of erythrocytes.

NOTE: The Sponsors expanded summary (Table 2, p 47) indicates that for full activity dog erythrocyte cytosol requires a heat labile, high molecular weight (greater than 300K) plasma component. The source of these data is stated. They were not found in the present study or any other Metabolism study.

The following purified enzymes or blood proteins failed to hydrolyze esmolol: Dog, Chicken, Rat or Human albumin, Human serum pseudocholinesterase, Human hemoglobin, Human erythrocyte carbonic anhydrase A or B, Human or Bovine erythrocyte acetylcholinesterase, and Electric eel acetylcholinesterase.

Species Difference in the Hydrolysis of Esmolol by Blood Esterase(s)

	In Vitro Half-Lives (min)		
Species	Whole Blood	Plasma	Washed Erythrocytes
Rat	2.5[a]	2.5[a]	98.5
Guinea Pig	2.5[a]	2.5[a]	102.
Dog	12.5	180.[a]	26.0
Human	27.2	180.[a]	60.4

[a] A half-life of 2.5 min indicates that more than 90% of the substrate was metabolized in 2. min. A half-life of 180 min indicates that less than 10% of the substrate was metabolized in 60 min.

Thus, whole blood from rats and guinea pigs had the highest level of activity while human whole blood had the lowest level of activity. In addition, most of the esmolol esterase activity in rats and guinea pigs was in the plasma but in dog and man it was in the erythrocyte.

Effects of Esterase Inhibitors on Esmolol Esterase Activity of Whole Blood

Inhibitor	Rat	Guinea Pig	Dog	Human
Echothiophate Iodide (4 mg/ml)	YES	YES	NO	NO
Eserine (10-5 M)	NO	NO	NO	NO
NaF (30 mg/ml)	YES	YES	YES	YES

YES = half-life prolonged more than 10 fold.
NO = half-line not prolonged.

Thus, these data indicate that the esmolol esterase in dog and man are similar but different than the esmolol esterase in rat and guinea pig.

The metabolism of esmolol by dog or human whole blood was not inhibited when incubated the following ester-containing agents at equal concentrations: Acetylcholine, Succinylcholine, Procaine or Chloroprocaine. Like wise, esmolol did not alter the metabolism of either Procaine or Chloroprocaine by dog or human whole blood.

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d. In vitro Metabolism of Esmolol by Human Blood:

In Vitro Half-Life (min) of Esmolol in Human Whole Blood

	Female	Male
Mean ± S.D	28.3 ±4.77	29.7 ±3.35
n	20	8

There was no difference in the esmolol esterase activity between Human female and male whole blood. In addition, unlike found in a rat teratology study, blood from human females did not indicate the presence of "fast" and "slow" esmolol esterase activity.

These studies indicate that neither acetylcholinesterase (located in erythrocyte membranes) of pseudocholinesterase (buterylcholicesterase) (located in plasma) are responsible for the hydrolysis of esmolol. Esmolol esterase is located in the cytoplasm of erythrocytes but is not hemoglobin, carbonic anhydrase or an esterase that is inhibited by eserine. The esterase may be similar to aspirin esterase which is also said to be located in the cytosol of human erythrocytes and is not inhibited by eserine. Failure of either echothiophate (organic phosphate) or eserine to inhibit dog or human esmolol esterase suggests that it may be an arylesterase (located in the erythrocyte cytosol). Esmolol should not interact with other esters which are metabolized by acetylcholinesterase or plasma cholinesterase as demonstrated for procaine, chloroprocaine, acetylcholine and succinylcholine.

e. Lack of Interaction Between Esmolol and Succinylcholine in Anesthetized Dogs: In separate groups of anesthetized (pentobarbital-barbital), vagotomized, artificially ventilated mongrel dogs, saline or esmolol was infused i.v. at 50 mcg/kg/min for 180 min during the i.v. infusion of succinylcholine at 0.04 mg/kg/min. Heart rate (HR) and blood pressure (BP) responses to i.v. isoproterenol (0.5 mcg/kg) remained constant during the 180 min of saline infusion and for 240 min after terminating the saline infusion. Infusion of esmolol during the ipfusion of succinylcholine caused the almost immediate reduction in the positive chronotropic but not the hypotensive action of isoproterenol. The inhibition of the HR effects of isoproterenol became stable with in 10-20 min and remained relatively constant throughout the 3 hr infusion period. By the end of the esmolol infusion period basal HR had decreased 14 ± 3 bts/min, not unlike that seen in other studies. The table below compares the beta-blocking action of esmolol observed in a previous study (ACC Central File No. 100-80-00450, Duration of beta-blocking action in anesthetized dogs) to that obtained in the presence of succinylcholine (present study).

	% Inhibition of HR effects of Isoproterenol	Time for Recovery From Blockade (min) 50%	80%
Esmolol + Succinylcholine (n = 5)	52 ±6	5 ±2	12 ±2
Esmolol (#100-80-00450) (n = 12)	56 ±6	6 ±1	12 ±3

Thus, succinylcholine had no effect on the beta-receptor blocking activity or the duration of activity of esmolol infused at 50 mcg/kg/min for 3 hours.

In another group of anesthetized dogs, succinylcholine was infused i.v. at 0.04 mg/kg/min and and artificially ventilation was started as soon as spontaneous respirations ceased. The infusion of succinylcholine continued for 60 min at which time the infusion was terminated and the time to spontaneous respiration was recorded. An infusion of either saline (6 dogs), esmolol (100 mcg/kg/min in 10 dogs or 300 mcg/kg/min in 9 dogs), or eserine (0.5 mg/kg over 5 min in 4 dogs) was started. Thirty minutes later the succinylcholine infusion - recovery was repeated. Inhibition of spontaneous respiration and initiation of artificial ventilation was associated with slight increases in basal heart rate and blood pressure. This effect was not prevented or enhanced by esmolol at either dose.

Time (min) to Spontaneous Respiration on Termination of Succinylcholine Infusion

Treatment (mcg/kg/min)	First Study n	Control	Treated	Second Study n	Control	Treated
Saline	6	31 ±5	38 ±5	5	38 ±5	39 ±4
Esmolol, 100	10	35 ±4	37 ±3	-	-	-
Esmolol, 300	9	47 ±4	60 ±7	6	43 ±3	44 ±11
Eserine, 0.5 mg/kg	4	41 ±1	+90	-	-	-

The slight non-significant increase in the succinylcholine recovery time in the 300 mcg/kg/min esmolol group was primarily due to prolongation seen in one animal. When this dog was eliminated from the analysis the recovery time from the control and treated succinylcholine infusions were 48 ±5 and 54 ±5 min, respectively. A second study failed to indicate that esmolol prolonged recovery from succinylcholine.

In summary, in contrast to eserine, the infusion of esmolol at doses up to 300 mcg/kg/min failed to prolong recovery from succinylcholine-induced respiratory paralysis.

f. Effect of Esmolol on the Hydrolysis of Benzoylcholine by Human Blood and Plasma:

Esmolol Concentration mcM	(mcg/ml)	% Inhibition of Benzoylcholine Hydrolysis by Human Plasma[1]	Benzoylcholine Half-Life (min) in Human Blood[2]
0.0	0.0	-	0.43 ±0.21
1.5	0.5	2.15 ±3.04	0.49 ±0.18
3.1	1.03	-	0.47 ±0.09
3.25	1.05	3.83 ±2.55	-
6.25	2.1	10.1 ±4.76	-
12.5	4.2	12.2 ±2.17	0.54 ±0.24
25	8.4	23.9 ±3.21	-
50	16.6	38.8 ±5.56	0.96 ±0.39
75	24.9	46.7 ±4.74	-
100	33.2	54.8 ±13.9	-
200	66.4	66.1 ±10.7	-
400	132.8	74.6 ±0.28	-
IC50		9×10^{-5}M (30 mcg/ml)	

Mean ±SD
1 Samples from 2-3 subjects.
2 Samples from 5 subjects.

Sponsor states that therapeutic concentrations of esmolol are 1-2 mcg/ml. Thus, at or below therapeutic concentrations esmolol had little or no effect of the hydrolysis of benzoylcholine by human plasma. Higher concentrations caused a concentration-dependent inhibition with and ID_{50} of 30 mcg/ml. Concentrations up to 4.2 mcg/ml had little effect on the whole blood half-life of benzoylcholine and caused only a 12% inhibition of hydrolysis by plasma. Benzoylcholine, like succinylcholine, is readily metabolized by plasma cholinesterase (pseudocholinesterase)

3. PROTEIN BINDING:

Esmolol and its Major Metabolite, ASL-8123:

Species or Protein Solution	Protein Conc. (g/dl)	% BOUND (mean) 1 Esmolol (20 mcM)	ASL-8123 (200 mcM)
House Serum	6.89	56.17	-
Rat Serum	5.67	44.31	2.50
Albumin[2]	(1.0 g%)	-	1.87
Rabbit Serum	5.39	41.96	8.67
Dog Serum	6.2	38.30	1.97
Human Serum	6.89	56.17	6.97
Albumin[2]	(4.0 g%)	24.45	4.30
AAG[3]	(66 mg%)	21.31	4.40

1 Samples were incubated 5 min in the presence of NaF to inhibit esterase activity. Equilibrium was achieved within 5 min and significant hydrolysis of esmolol occurred in 60 min. Protein binding was determined by ultrafiltration and HPLC-UV. Each value is the mean of 5-7 samples. Esmolol at 20 mcM is 6.63 mcg/ml.

2 Albumin solutions were made in physiologic isotonic buffer.

3 AAG = a_1-Acid Glycoprotein in physiologic isotonic buffer.

Neither Esmolol at 9.9 to 385 mcM (3.28 to 127.6 mcg/ml) of ASL-8123 at 35.5 to 355 mcM demonstrated concentration-dependent binding to AAG (13 to 26% bound) or rat serum (8.5 to 15.9% bound), respectively.

4. DISTRIBUTION AND EXCRETION:

a. Mass Balance Study in Rats: Urinary and fecal excretion of 14C-labeled material was measured from rats which received 14C-labeled ASL-8052 administered i.v. as: 1) a single dose (30 mg/kg); 2) multiple injections (12 mg/kg q 2h) for 10 hours; or 3) an infusion (100 mcg/kg/min) for 10 hours. Urine and feces were collected for up to 48 hours after the single dose and for up to 45 hours and 47 hours after the end of the 10 period of infusion or multiple injection, respectively.

Total urinary and fecal recovery of labeled material was 93%, 101% and 100% with single dose, infusion and multiple dose administration, respectively. From 97% to 99% of the total was recovered within the first 24 hours and 74% of the total was recovered in the first 4 hour after the single dose and 64% to 69% in 6 hours after the infusion or multiple doses.

About 87% (single dose) to 95% (infusion and multiple doses) of the administered dose was recovered in the urine with less than 5% appearing in the feces. Therefore, biliary excretion was very slight.

HPLC analysis of the 24 hour urine sample indicated the presence of 6 compounds. Two compounds were identified as parent ASL-8052 (5% of administered dose) and ASL-8123 (77% of administered dose). The other 4 compounds were unidentified. The similarity of results with infusion, multiple dose and single dose suggests that the enzyme system was not saturated by the multiple dose or infusion schedule.

b. Distribution of Esmolol and its Acid Metabolite in Rats: Thirty-six male Sprague Dawley rats received (under ether anesthesia) 14C ASL-8052, 30 mg/kg i.v. (tail vein) as a bolus. Blood , tissues and organs were obtained from 3 sacrificed rats (under ether anesthesia) at each of the following time intervals after dosing: 5, 10, 15, 20, 30, 45, 60, 90, 120, 180, 240, and 420 min. Total labeled material, parent ASL-8052 and its major metabolite, ASL-8123, were measured at each time interval in blood, brain, heart, kidneys, liver, lung, spleen, testes, and GI tract; and at 5, 10 and 420 min in skin and carcass.

	% of Labeled Material		ASL-8052 ESMOLOL		ASL-8123 METABOLITE	
	Max	Time(min)	Cmax	Time(min)	Cmax	Time(min)
Blood	4.97	15	3.26	5	23.5	20
Heart	0.57	5	14.8	5	38.8	5
Kidneys	6.51	5	0.98	5	258.	5
Liver	9.17	15	0.41	5	95.2	45
Lung	1.94	5	-	-	-	-
Brain	0.53	5	18.4	5	23.6	20
Spleen	0.44	15				
Testes	0.29	15				
GI Tract	8.71	15				
Skin	8.88	5				
Carcass	51.0	10				
Total	88.1	5				

Cmax = mcg/ml or mcg/g

HALF-LIFE and AUC in the RAT for ESMOLOL and its MAJOR METABOLITE

	ASL-8052		ASL-8123	
	t1/2	AUC	t1/2	AUC
Blood	12.0	24.9	89.6	2761
Brain	7.03	189.	203.	4746
Heart	6.72	130.	141.	5501
Kidneys	11.1	11.3	77.0	18386
Liver	10.0	3.08	115.	14442

t1/2 = half-life, min.

AUC = area under curve, mcg/min/ml or gm.

Within 5 min of administration of 14C-esmolol, labeled material (which included both parent compound and metabolite) was found in all tissues and organs examined; peak levels occurred in 5 to 15 min. Amounts ranging from 2% to 9% of the administered label were found (arranged from highest to lowest) in the liver, GI tract , kidneys , blood, and lung . Less than 1% of the labeled material was found in the heart, brain, spleen and testes. The remaining carcass plus the skin contained about 59% of the administered dose. At 5 and 10 min 88.1% and 87.5% of the doses, respectively, was accounted for. This declined to 14.9% at 420 min (7 hrs). In all tissues, at all time points, most of the labeled material was the metabolite of Esmolol. The tissue:blood ratio for total radioactivity was higher than 1 in all tissues, except testes, suggesting rapid accumulation by tissues. The tissue:blood ratio for esmolol at peak blood levels, as well as at later time intervals, was greater than 1 except in the kidneys and liver. In contrast, the tissue:blood ratio of the acid metabolite was greater than 1 in all tissues and was highest in kidney and liver. The blood and tissue concentrations of esmolol were lower than those of its metabolite indicating rapid hydrolysis. Thus, the ratio of acid metabolite:esmolol was greater than 1 in all tissues and reached very high levels in the kidneys and liver. This condition could indicate that the kidney and liver were major sites of metabolism of esmolol or that these tissues have a high affinity for the metabolite generated by hydrolysis of esmolol in blood. It should be noted that previous studies in the rat indicate that 80% of administered esmolol was excreted by the kidney as the acid metabolite with less than 5% of the parent drug was recovered in the urine. The rate of elimination of esmolol from blood and tissues was rapid with a half-life of from 7 to 12 min and followed first order kinetics. The elimination from blood, kidney and liver was about equal and faster than in brain and heart. Based on both half-lives and AUCs the rate of elimination of the acid metabolite from blood and tissues was much slower than for esmolol. Esmolol rapidly entered the brain achieving concentrations that were more than 10 times higher than blood levels. In contrast, the levels of the acid metabolite in brain and blood were about equal, although levels of the acid metabolite in the brain were higher (about 2 X) than levels of esmolol.

5. Rate of Esmolol Infusion, Plasma Levels, beta-Receptor Antagonism, Clearance, Volume of Distribution and Half-Life in Dogs:

ASL-8052 was infused i.v. at increasing doses of 25, 50 and 100 mcg/kg/min to 9 anesthetized (pentobarbital/barbital) dogs (3 beagle and 6 mongrel) of either sex. Each infusion lasted for 60. Heart rate responses to isoproterenol, 0.5 mcg/kg, were determined before, at 10 min intervals during each infusion, and and for 1 hr after terminating the highest infusion rate. Blood samples were also obtained during each infusion and for 45 min after the highest infusion.

		Steady State ASL-8052 Blood Levels (mcg/ml)	% Inhibition of Heart RAte Responses to Isoproterenol	Clearance* (mcg/kg/min)	Volume* Distribution (l/kg)	T1/2* (min)
25 mcg/kg/min						
Mongrel	(6)	0.093	43.1	-	-	-
Beagle	(3)	0.140	41.9	-	-	-
Combined	(9)	0.105	42.7	238	-	-
50 mcg/kg/min						
Mongrel	(6)	0.200	58.8	-	-	-
Beagle	(3)	0.303	54.6	-	-	-
Combined	(9)	0.236	57.4	212	-	-
100 mcg/kg/min						
Mongrel	(6)	0.463	68.7	-	-	-
Beagle	(3)	0.573	64.1	-	-	-
Combined	(9)	0.501	67.2	200	4.07	1.19

* Clearance, volume of distribution and T1/2 were determined in 3 mongrel and 3 beagle dogs.

These studies were initially performed with mongrel dogs but the coefficient of variation of the blood concentrations averaged 80%. Therefore, beagle dogs were also studied. Their coefficient of variation of blood levels was only about 17% and blood levels were consistently higher than in mongrel dogs. These differences blood levels of ASL-8052 between beagle and mongrel dogs was not reflected in differences in pharmacologic effect (beta receptor blockade).

Steady state blood levels were obtained within 30 minutes and maximal beta blockade was achieved within 10 min of initiating each infusion. there was a linear relationship between the steady state blood level and the degree of beta receptor blockade.

Blood levels of ASL-9052 decreased very rapidly after stopping the infusion and decreased to less than 10% (below detectable limits) of steady state levels within 15 min in all dogs. Although it appeared that ASL-8052 disappearance slightly more slowly from mongrel than beagle dogs this difference was not certain since only 3 animal in each strain were sampled during recovery. Recovery from beta-receptor blockade was also very rapid with full recovery in 6/9 animals in 20 min. The 3 animals not fully recovered in 20 min were mongrel dogs.

6. Pharmacokinetics of Esmolol in Dogs: Conscious, male, mongrel dogs received 14C-ASL-8052 either as a single i.v. bolus of 10 mg/kg* (3 dogs) or by i.v. infusion at 200 mcg/kg/min for 12 hours (4 dogs). Levels of 14C-8052 and its major acid metabolite, 14C-ASL-8123, were determined in blood and urine samples. Urine samples were collected hourly. In the i.v. bolus study blood samples were collected for up to 6 hours (360 min). In the i.v. infusion study blood samples were collected during the 2 hr infusion and for 8 hours (480 min) after the infusion was terminated.

*NOTE: Methods section states bolus dose as 30 mg/kg but summary, tables and figures indicate dose was 10 mg/kg.

After the i.v. bolus injection the blood levels of parent compound disappearance biexponentially according to a two-compartment model. The acid metabolite, on the other hand, disappearance monoexponentially consistent with a one-compartment model. When ASL-8052 was infused at a constant rate of 200 mcg/kg/min, steady state plasma levels of 0.632 ±0.2 mcg/ml were attained within 20 min.

	ASL-8052		ASL-8123	
	Bolus	Infusion	Bolus	Infusion
Steady State Conc., mcg/ml	-	0.632		
Time of Steady State, min		20.		
Peak Concentration, mcg/ml	-	0.595	6.89	16.1
Peak time, min	-		32.7	126.
Distribution Half-Life, min	3.59	1.26*	-	-
Elimination Half-life, min	51.4	41.2	112.	106.
Volume of Distribution, l/kg	27.5	20.10	1.04	0.79
Central Compartment Vol., l/kg	3.03	1.15*		
Steady State Volume of Distribution	12.09	10.21		
Renal Clearance, ml/kg/min	1.56	1.93		
Total Body Clearance, ml/kg/min	370.	336.	6.47	5.16
AUC, mcg/ml/min	27.5	75.9	1508.	4378.
Urinary Recovery, % dose	0.41	0.54	57.0	69.3
0-8 hr Urinary Flow, ml/min	0.069	-	-	-
0-8 hr Creatinine Cl., ml/min/kg	2.37	-	-	-

* Significantly different from Bolus.

The elimination half-life of ASL-8052 (41-51 min) after bolus or infusion in the dog was 4 to 5 times longer that reported in humans (9.19 ± 3.51 min).

Renal clearance of unchanged compound was only about 0.5% of total body clearance indicating extensive and rapid metabolism in the dog. In support of this, only about 0.5% of the dose was recovered in the urine as unchanged ASL-8952 while 57-69% of the doses was recovered as the metabolite, ASL-8123.

The elimination of the metabolite, ASL-8123, was much slower than that of the parent compound (longer elimination half-life, slower total body clearance and much greater AUC). In addition, peak plasma levels of the metabolite did not occur until about 30 min after the bolus administration and 6 min after the termination of the infusion.

The total body clearance of ASL-8052 was 10 time the reported normal hepatic blood flow in dogs (37.5 ml/kg/min).

The volume of distribution of the parent compound was 34 to 46 times larger than the reported total body water in dogs (0.6 l/kg). This coupled with the observation that the parent compound is not extensively bound to plasma proteins and the volume of distribution is larger than the central compartment indicate preferential tissue uptake of the compound.

Peak plasma levels of the metabolite were 40 times higher than levels of the parent compound. In addition, the volume of distribution of the metabolite was much smaller than that for the parent compound and only 1.3 to 1.7 times total body water (0.6 l/kg).

7. OCULAR ADMINISTRATION:

a. Systemic Absorption in the Rabbit: New Zealand white rabbits (4) received 14C-ASL-8052, 3 mg (100 mcl of a 3% solution), either i.v. or applied to the right eye according to a cross-over design (5 days separated treatments). Blood was collected at 2.5 to 240 min after each administration and analyzed for total radioactive material, ASL-8052 and ASL-8123.

	ROUTE OF ADMINISTRATION OF 14C-ASL-8052			
	OCULAR		I.V.	
	ASL-8052	ASL-8123	ASL-8052	ASL-8123
Peak Concentration, ng/ml	24.9	246	-	1000
Peak Time, min	2.5*	45	-	15
T1/2, min	5.60	105	4.95	78.5
AUC, ng/ml/min	220	25820	1305	111624

* less than 2.5 min.

In the rabbit ASL-8052 instilled into the eye is rabidly absorbed systemically reaching peak plasma levels in less than 2.5 min. The area under the blood-time curve of both ASL-8052 and ASL-8123 indicate that about 17% of the ocular dose was absorbed systemically.

b. Ocular Distribution - Ocular Administration - Rabbits: New Zealand white rabbits received 50 mcl of a 1% ASL-8052 solution in both eyes (50 mcg/eye) and 3 rabbits were sacrificed for analysis of 14C-ASL-8052 and 14C-ASL-8123, its acid metabolite.

CONCENTRATION OF 14C-ASL-8052 and 14C-ASL-8123 (ng/100 mcl or ng/50mg)

TIME	AQUEOUS HUMOR		CORNEA		IRIS-CILIARY BODY	
(min)	ASL-8052	ASL-8123	ASL-8052	ASL-8123	ASL-8052	ASL-8123
2.5	9.49	2.24	389	3465	11.6	73.1
5.0	74.5	6.81	971	5155	29.8	137.
15.0	37.4	55.7	343	5661	22.6	165.
30.0	19.4	163.	138	5284	23.1	295.
60.0	6.0	217.	63.0	3980	7.80	122.
120.0	1.17	293.	26.2	2597	3.90	96.0
240.0	0.0	220.	17.7	1768	2.29	85.5

ASL-8052 applied to the cornea of rabbits was rapidly absorbed reaching peak levels in the cornea, aqueous humor and iris-ciliary body in 5 min. The disappearance of ASL-8052 was rapid with calculated half-lives in the aqueous humor, cornea and iris-ciliary body of 15 min, 15 min and 30 min, respectively.

The peak concentrations of the acid metabolite, ASL-8123, were much higher and occurred 10 min (cornea) to 115 min (aqueous humor) later than for the parent compound. The elimination of ASL-8123 was much slower than that of ASL-8052

The conclusion that the cornea and iris-ciliary body but not aqueous humor rapidly hydrolyze ASL-8052 is supported by the fact that the ratio of ASL-8123:ASL-8052 was greater than 1 in cornea and iris-ciliary body at 2.5 min (earliest time point) but not until 15 min in aqueous humor. Thus ASL-8052 is rapidly metabolized during ocular absorption.

c. Ocular Distribution - Ocular and I.V. Administration - Dogs: 14C-ASL-8052, 600 mcg was administered intra-ocularly (100 mcl of a 3% solution to each eye or 300 mcg to each eye) to 10 mongrel dogs. Two dogs were sacrificed at 15, 30, 60, 120 and 240 min for tissue analysis. Blood samples were obtained at the time of sacrifice and in the 240 min dogs, blood samples were obtained at 0.5 min to 240 min. In 2 other mongrel dogs the same total dose (600 mcg) was administered i.v. and blood samples were collected from 0.5 min to 240 min after administration.

CONCENTRATION OF 14C-ASL-8052 and 14C-ASL-8123
IN EYE STRUCTURES AFTER INTRA-OCULAR
ADMINISTRATION OF 14C-ASL-8052

(ng/100 mcl or ng/50mg)

TIME	AQUEOUS HUMOR		CORNEA		IRIS-CILIARY BODY	
(min)	ASL-8052	ASL-8123	ASL-8052	ASL-8123	ASL-8052	ASL-8123
15	5.07	29.6	186	2383	80.2	20.8
30	7.58	72.9	193	2109	63.0	17.9
60	4.03	197.	207	3241	123.	64.2
120	3.39	240.	144	1927	149.	58.1
240 (IO)	1.36	143	46.9	791	130.	40.3
240 (IV)	-	0.95	-	1.10	22.6	1.46

BLOOD LEVELS

	ROUTE OF ADMINISTRATION OF 14C-ASL-8052			
	OCULAR		I.V.	
	ASL-8052	ASL-8123	ASL-8052	ASL-8123
Peak Concentration, ng/ml	5.55	121	173	416
Peak Time, min	1.0	60	1.0	24
T1/2, min	5*		5*	
AUC, ng/ml/min	17.12	20262	898.9	57645

* Stated by sponsor to be less than 5 min.

	ELIMINATION HALF-LIFE, T1/2 (min)	
TISSUE	ASL-8052	ASL-8123
Aqueous Humor	93.4	160
Cornea	82.5	90
Iris-Ciliary Body	472.	260
Blood*	4.5	130

* Although not specifically stated, sponsor suggests that the half-lives in blood were derived from the combined intra-ocular and i.v. dosing studies.

After intra-ocular administration of ASL-8052 the parent compound and its metabolite, ASL-8123, were found in the cornea, iris-ciliary body and aqueous humor in 15 min and in blood in 30 sec. Peak levels occurred in eye structures at 30 to 120 min and in blood at 5 min for ASL-8052 and 60 fon for ASL-8123.

The concentration ratio of ASL-8123:ASL-8042 was greater than 1 at all time points in the aqueous humor and cornea suggesting metabolism of ASL-8052 to ASL-8123. In contrast, ASL-8052 appeared stable in the iris-ciliary body since the concentration ratio of ASL-8123:ASL-8052 was always less than 1.

Compared to results of in vitro studies, the shorter half-life of ASL-8052 in eye structures found in this in vivo study is probably related to the destruction of esterase activity by the homogenizing process used in the in vitro studies.

At 240 min after i.v. administration ASL-8052 was found in the iris-ciliary body (22.6 ng/50 mg) but not in the aqueous humor or cornea; low levels (less than 2 ng/50 mg or 100 mcl) of ASL-8123 were found in the eye structures.

Based on the AUCs of i.v. and intra-ocular administration, the systemic bioavailibility of ASL-8052 after ocular administration was estimated to be 1.9% and that of ASL-8123 was about 35%. These are only estimates since loss of drug due to tearing and overflow were not accounted for.

Sponsor states that the half-lives of ASL-8052 and ASL-8123 in dog blood were very short and independent of route of administration.

C. TOXICOLOGY:

1. Submitted Studies: Studies performed by American Critical Care, McGaw Park , IL (STUDY #, Title, study dated & location):

T8052-001: Acute Intravenous Toxicity of ASL-8052 in mice, 3/27/80 to 11/6/80. Vol 1.2, p. 44-56.

T8052-002: Acute Intravenous Toxicity of ASL-8052 in rats. 9/3/80 to 11/6/80, vol. 1.2, p. 57-68.

T8052-003: Acute Intravenous Toxicity of ASL-8052 in Dogs. 9/25/80 to 10/9/80, vo. 1.2, p. 80-91.

T8052-004: Acute Toxicity of ASL-8052 by short intravenous infusion in dogs. 10/15/80 to 2/27/81, vol 1.2, p. 92-102.

T8052-005: Subacute Intravenous dose-ranging of ASL-8052 in rats. 11/04/80 to 11/11/80, vol. 1.2, p. 120-149.

T8052-006: Subacute Dose-ranging study by continuous infusion of ASL-8052 in Beagle dogs, 12/04/80 to 12/12/80, vol. 1.2, p. 150-213.

T8052-011: Acute Intravenous toxicity (LD50) of an ASL-8052 formulation and vehcile in male and femal mice. 12/16/80 to 12/31/80, vol. 1.2, p. 103-119.

T8052-012: Seven Day Continuous Intravenous Infusion Study of ASL-8052 in BEagle dogs. 06/03/81 to 06/24/81, vol. 1.4, p. 1-115.

T8052-013: Pharmacodynamic Study of ASL-8052 in the rat. 12/08/80 to 04/20/81, vol. 1.4, p. 116-135.

T8052-014: Acute Intravenous Toxicity (LD50) of ASL-8052 compared to the Acid metabolite (ASL-8123) in male and female mice. 01/13/81 to 01/16/81, vol. 1.4, p. 136-146.

T0852-015: Determination of the maximum tolerated dose of ASL-8052 in rabbits. 01/19/81 to 01/24/81, vol. 1.2, p. 69-79.

T8052-016: Perivascular and Intravenous Irritation Testing of an ASL-8052 Formulation in New Zealand White Rabbits. 02/02/81 to 02/26/81, vol. 1.4, p. 147-208.

T8052-017: In vitro effects of an ASL-8052 Formulation (100 mg/ml) on human blood. 02/20/81 to 02/21/81, vol. 1.4, p. 209-217.

T8052-020: Acute Ocular Irritation Screen of ASL-8052 in New Zealand White Rabbits (ACC # 100-82-00004; Report date: none; Study dates: 11/23/81 to 11/30/81; QAUIR - none; vol. 1.5; p. 255-277).

T8052-022: Acute Ocular Irritation Evaluation of a Formulation of ASL-8052 in New Zealand White Rabbits (ACC, McGraw, Il; Report date: none; ACC # 100-82-?; QUAIR dated 08.19/82; Study dates: 4/6/82 to 4/13/82; vol. 1.5, p 347-384).

T8052-023: Acute Oral Toxicity of ASL-8052 in Rats (ACC, McGaw Park, IL, Central File No. 100-82-01139; Report not dated; Study dates: 02/02/82 to 02/18/82; QUAIR dated 10/13/82; vol. 1.5, p 385-400).

T8052-024: Acute Intravenous Toxicity of an Aqueous Formulation of ASL-8052 in Rats (ACC, McGaw, IL.; Central File # 100-82-01286; Report not Dated; Study dates: 03/24/82 to 04/07/82; QUAIR dated 11/24/82; vol. 1.5, p. 401-414)

T8052-027: In-Vitro Effect of an ASL-8052 Formulation on Human Blood (ACC. McGaw Park, IL,; Central Files No. 100-82-01106; Report not Dated; Study Dates 04/13/82 to 04/14/82; QUAIR dated 10/08/84; vol. 1.6, p. 1-10)

T8052-028: Evaluation of ASL-8052 for Local Anesthetic Activity on New Zealand White Rabbits (ACC, McGaw Park, IL; Central File No. 100-52-00517; Report Not Dated; Study Dates: 03/17/82 to 03/18/82; No QAUIR; vol. 1.6, p. 11-22)

T8052-031: Local Effects of an ASL-8052 Formulation Infused Directly into the Jugular Vein of Beagle Dogs for Three Days. (ACC, McGaw Park, IL; Central File No. 100-83-01169; Report not dated; Study Dates 05/06/83 to 05/09/83; QAUIR 09/29/83; vol. 1.6, p. 91-106)

T8052-032: Local Effects of an ASL-8052 Formulation Infused into a Central Vein of Beagle Dogs for 24 or 48 Hours. (ACC, McGaw, IL, Central File No. 100-83-01733; Report not dated; Study dates 06/01/83 to 06/03/83; QAUIR 11/11/83; vol 1.6, p 107-129)

T8052-035: Local Effects of an ASL-8052 Infused into the Jugular Vein of Dogs for 72 Hours. (ACC, McGaw Park, IL; Central File No. 100-83-01747; Report not dated; Study Dates 07/26/83 to 08/05/83; QAUIR 11/22/83; vol. 1.7, p. 1-21)

T8052-036: Comparison of the Acute Intravenous Toxicity of levo-ASL-8052 and dl-ASL-8052 in Mice. (ACC, McGaw Park, IL; Central File No. 100-84-00081; Report not dated; Study Dates 09/14/83 to 09/28/83; QAUIR 02/10/84; vol. 1.7, p. 22-36)

T8052-037: Comparison of the Acute Intravenous Toxicity of levo-ASL-8052 and dl-ASL-8052 in Rats. (ACC, McGaw Park, IL; Central File No. Not Legible; Report not dated; Study Dates 09/22/83 to 10/06/83; QAUIR 03/05/84; vol. 1.7, p. 37-49)

T8052-038: Comparison of the Venous and Perivascular Irritation of Two ASL-8052 Formulations in Rabbits. (ACC, McGaw Park, IL; Central File No. None; Report not dated; Study Dates 10/18/83 to 10/27/83; QAUIR 05/31/84; vol. 1.7, p. 50-73)

T8052-039: Comparison of the Acute Intravenous Toxicity of Two ASL-8052 Formulations in Rats. (ACC, McGaw Park, IL; Central File No. 100-84-00486; Report not dated; Study Dates 11/08,09/83 to 11/22,23/83; QAUIR 03/06/84; vol. 1.7, p. 74-85)

T8052-040: In Vitro Effect of an ASL-8052 Formulation on Human Blood. (ACC, McGaw Park, IL; Central File No. Not Legible; Report not dated; Study Dates 11/02/83 to 11/03/83; QAUIR 03/06/84; vol. 1.7, p. 86-96)

T8052-041: Comparison of the Venous and Perivascular Irritation of levo-ASL-8052 and dl-ASL-8052 in Rabbits. (ACC, McGaw Park, IL; Central File No. None; Report not dated; Study Dates 11/29/83 to 12/08/83; QAUIR 06/04/84; vol. 1.7, p. 97-119)

T8052-042: Venous and Perivascular Irritation of ASL-8052 Formulation Components in Rabbits. (ACC, McGaw Park, IL; Central File No. None; Report not dated; Study Dates 12/13/83 to 12/22/83; QAUIR 06/04/84; vol. 1.7, p. 120-145)

T8052-043: Determination of the Effect of Infusion Volume Rate on Venous Irritation Produced by a ASL-8052 Formulation in Rabbits. (ACC, McGaw Park, IL; Central File No. None; Report not dated; Study Dates 01/30/84 to 02/03/84; QAUIR 06/04/84; vol. 1.7, p. 146-162)

T8052-044: Acute Toxicity of levo-ASL-8052 and dl-ASL-8052 in Dogs by Short Intravenous Infusion. (ACC, McGaw Park, IL; Central File No. None; Report not dated; Study Dates 03/19/84 to 04/09/84; QAUIR 06/04/84; vol. 1.7, p. 163-171)

Studies performed by International Research and Development Corporation, Mattawan, MI. Blood samples were analyzed for ASL-8052 and ASL-8123 by American Critical Care.

T8052-007 (IRDC # 189-010): Fourteen-Day Intravenous Toxicity Study of ASL-8052 in Rats. 2/11/81 to 2/25/81, vol 1.2, p. 214-361.

T8052-008 (IRDC# 189-011): Fourteen-Day Continuous Intravenous Infusion Study of ASL-8052 in Beagle Dogs, 02/06/81 to 03/12/82, vol. 1.3, p. 1-363.

T8052-029: Dose Range-Finding Teratology Study in Rabbits (IRDC, Mattawan, MI. Report No. 189-019 Dated Nov. 17, 1983. ACC Central File No. 100-84-00695; GLP-QAS dated 11/16/83. Vol. 1.6, p. 23-54.

T8052-030: Dose Range-Finding Teratology Study in Rats (IRDC, Mattawan, MI. Report No. 189-017, dated Sept. 8, 1983; GLP-QAS dated Sept. 8, 1983. ACC Central File NO. not indicated. Vol. 1.6, p. 55-90.

T8052-033: Teratology Study in Rats (IRDC, Mattawan, MI. Report No. 189-018, dated FEb. 29, 1984; IRDC GLP-QAS dated Feb. 29, 1984. ACC QAUIR dated May 5, 1934. ACC Central File no. not indicated. vol. 1.6, p. 130-227)

T8052-034: Teratology Study in Rabbits (IRD, Mattawan, MI. Report No. 189-020, dated Apr. 26, 1984; IRDC GLP-QAS dated Mar. 05, 1984 and ammended Apr. 25, 1984. ACC report on blood levels dated Apr., 1984 and QAUIR dated Apr 24, 1984; ACC QAUIR dated May 11, 1984. ACC Central File NO. not indicated. vol. 1.6, p. 228-332)

T8052-021: A Three-Week Eye Irritation Study of ASL-8052 in New Zealand White Rabbits (Bio-Research Laboratories Ltd., Senneville, Quebec, Canada, No. 50148 dated Feb. 9, 1983; ACC # 100-84-00394; Study dates: 5/17/82 to 7/2/82; GLP-QA 2/22/83; Vol. 1.5, p. 278-346).

T8052-045: The Effect of Esmolol vs. Placebo on Blood Coagulation and Platelet Function. (Dr. Ronald G. Strauss & Associates, University of Iowa Hospital and Clinics, Iowa City, Iowa; Central File No.: None; Report not dated; Study Dates 12/15/83 to 12/23/83; QAUIR: None; vol. 1.7, p. 172-186)

2. Acute Toxicity:

a. Intravenous: The acute effects of intravenous (i.v.) ASL-8052 were determined in mice, rats, rabbits and dogs in studies performed byb the sponsor. These studies included: 1) hemodynamic effects in anesthetized rats; 2) LD50 and signs of toxicity in mice, rats, rabbits and dogs; 3) the influence of administration rate on manifestations of toxicity in dogs; 4) a comparison, in the mouse, of the toxicity of ASL-8052 in saline with its acid metabolite (ASL-8123), with methyl alcohol (a by product of its metabolism) and with ASL-8052 in 20% propylene glycol/20% alcohol formulation ; 5) a comparison of the acute i.v. toxicity of levo-ASL-8052 with dl-ASL-8052 in the mouse, rat and dog; 6) a comparison of the acute i.v. toxicity of two ASL-8052 formulations (10% propylene glycol/10% alcohol and 20% glycerin /60% alcohol) in the rat; and 7) intravenous and perivascular irritation in the rabbit and dog.

(1) Hemodynamic Effects in Anesthetized Rats: In urethane anesthetized rats (n=7) i.v. ASL-8052 caused dose-related decreases in heart rate, mean blood pressure, pulse pressure, ECG amplitude and respiratory rate; the minimum effective (a 12.5% decrease) doses for these effects were 1.5, 2.3, 2.6, 5 and 24.7 mg/kg respectively. Tachyphylaxis did not occur and effects lasted less than 2.5 min at doses up to 10 mg/kg i.v. The mean lethal dose was 50 mg/kg and doses between 40 and 80 mg/kg usually caused abrupt respiratory arrest followed by circulatory collapse. Death could frequently be prevented by respiratory support. In a small percentage of animal circulatory collapse proceeded respiratory arrest. The cause (respiratory or cardiovascular) of death in other studies using conscious rats, mice, rabbits or dogs was not indicated. Effects of ASL-8052 on heart rate, blood pressure and pulse pressure but not on ECG amplitude, respiratory rate of the lethal dose, depends on the level of sympathetic tone since hexamethonium, a ganglionic blocking agent, reduced the magnitude of effects on the former but not the latter functions.

(2) LD50 and Signs of Toxicity in Mice, Rats, Rabbits and Dogs: In mice (10/sex/dose), rats (10/sex/dose), and dogs (2/sex/dose a lethal i.v. dose (single administration with a 14-day observation) caused death on the 1st day, usually within minutes of dosing, and there were no differences between males and females. The combined (males & females) LD50's in mice, rats and dogs were 92.9, 70.9 and 31.6 mg/kg, respectively. In rabbits (6/sex) receiving increasing single daily i.v. doses on consecutive days, the LD50 was approximately 40 mg/kg. No drug related gross pathology was noted at necropsy in either rats, dogs or rabbits dying on study or in survivors.

Signs of toxicity observed in mice, rats, rabbits and dogs were similar and included dyspnea, prostration, clonic/tonic convulsions, hypokinesia, hyperpnea, ataxia, sedation and tremors. Rabbits and dogs also had constricted or dilated pupils. White rabbits showed signs of vasoconstriction, dogs vocalized, urinated, defecated, salivated, vomited and had ptosis.

(3) The Influence of Administration Rate on Manifestations of Toxicity in Dogs: The toxicity of ASL-8025 in the dog was greatly reduced when infused at a slow rate. When the doses of the compound were administered over one minute intervals, the LD50 was 31.5 mg/kg (2/sex/dose). However, no deaths occurred when a dose of up to 240 mg/kg was infused over a 60 min interval (1/sex). In addition, at this slow rate of administration, no behavioral effects were observed at up to 120 mg/kg and 180 mg/kg caused only salivation and some ataxia.

(4) A Comparison in the Mouse of the Acute Toxicity of ASL-8052 in Saline with its Acid Metabolite (ASL-8123), with Methyl Alcohol (a product of ASL-8052 metabolism), and with ASL-8052 in a 20% Propylene Glycol / 20% Alcohol Formulation: In studies using mice (10/sex/dose), the LD50 of ASL-8052 was essentially the same when dissolved in saline (55.1 mg/kg) as when dissolved in the proposed vehicle (55.5 mg/kg) but 6.9 times less than ASL-8123 (452 mg/kg), the acid metabolite. Methyl alcohol, a metabolic by-product of ASL-8052, was nontoxic at 80 mg/kg i.v. This dose is about 1.3 times the amount of methyl alcohol expected at a ASL-8123 dose of 582 mg/kg, the highest dose tested.

In rabbits (6/sex), plasma levels of ASL-8052 or ASL-8123 measured within 10 minutes of i.v. administration were directly related to dose. These studies also indicate that ASL-8052 is rapidly metabolized to ASL-8123 and that at 40 mg/kg blood levels of both ASL-8052 and ASL-8123 were substantially higher in animals that died (n=6; 27.1 & 77.4 mcg/ml, respectively) than in animals that survived (n-6; 4.21 and 34.7 mcg/ml, respectively).

(5) A Comparison of the Acute i.v. Toxicity of levo-ASL-8052 with dl-ASL-8052 in the Mouse, Rat, and Dog:

	MOUSE	
	MALE	FEMALE
l-ASL-8052		
Doses Tested, mg/kg	50, 60, 80, 100. 130, 160	
LD50, mg/kg (95% C.L.)	123.0 (110.2-137.0)	150.6 (137.5-171.7)
Combined LD50, mg/kg (95% C.L.)	136.5 (126.9-148.2)	
Highest Non-Lethal Dose, mg/kg	80	100
Lowest Lethal Dose, mg/kg (#dead/#tested)	100 (1/10)	130 (1/10)
Total Deaths/# Given Lethal Doses	17/30	8/30
Day of Deaths	Day 1: 17/17	8/8
	All deaths occurred within 10 min of dosing. There were 3 and 1 surviving males in the 130 and 160 mg/kg groups, respectively and 3 surviving females in the 160 mg/kg group.	
dl-ASL-8052		
Doses Tested, mg/kg	50, 60, 80, 100. 130, 160	
LD50, mg/kg (95% C.L.)	109.5 (92.9-135.2)	129.1 (114.9-147.4)
Combined LD50, mg/kg (95% C.L.)	119.1 (90.6-203.4)	
Highest Non-Lethal Dose, mg/kg	60	80
Lowest Lethal Dose, gm/kg (#dead/#tested)	80 (4/10)	100 (1/10)
Total Deaths/# Given Lethal Doses	21/40	15/30
Day of Deaths	Day 1: 21/21	15/15
	All deaths occurred within 10 min of dosing. There were 3 and 1 surviving males in the 130 and 160 mg/kg groups, respectively and 4 and 2 surviving females in the 130 and 160 mg/kg group, respectively. The death of one male in the 50 mg/kg group on day 6 was not considered drug-induced.	

Separate groups of 10 mice (CD-1)/sex/dose received a single dose of either l-ASL-8052 (lot No. 907-40) or dl-ASL-8052 (lot No. 907-37) intravenously (tail vein). Mortality, signs of toxicity, and body weight were recorded over a 14 day observation period. ASL-8052 was dissolved in appropriate volumes of 0.9% Sodium Chloride, USP. Each dose was administered in a volume of 20 ml/kg at a rate of 0.5 ml/min (controlled by a syringe pump).

Signs of Toxicity were seen at all doses and were dose-related. Tremors were seen at all dose levels with both compounds. Effects seen in males at 60 mg/kg and above and in females at 80 mg/kg and above also included ataxia, sedation, hyperpnea, dyspnea, prostration and clonic convulsions. The last 3 signs were most prevalent at lethal doses. Hyperpnea was the least frequent sign observed. In survivors, all toxic signs disappeared in 10 minutes and weight gains of all groups were similar.

NOTE: The Sponsor does not indicate signs of toxicity in animals which died. The most common signs at doses which did not cause mortalities were tremors at the lowest dose, and sedation and ataxia at the maximum non-lethal doses. The most common effects seen at lethal doses were clonic convulsions, prostration and dyspnea.

Summary: In mice, the signs of acute i.v. toxicity of levo-ASL-8052 and dl-ASL-8052 were essentially the same and included ataxia, sedation, hyperpnea dyspnea, prostration and clonic convulsions. In survivorsm all toxic signs disappearance in 10 minutes. The highest non-lethal dose of levo-ASL-8052 was 80-100 mg/kg while that for dl-ASL-8052 was 60-80 mg/kg. The LD_{50} tended to be slightly higher foe levo-ASL-8052 than for dl-ASL-8052 (combined LD_{50}: 136.5 mg/kg vs 119.1 mg/kg). All deaths occurred within 10 minutes of dosing.

(6) Rat:

	RAT	
	MALE	FEMALE
l-ASL-8052		
Doses Tested, mg/kg	45, 60, 70, 85	
LD50, mg/kg (95% C.L.)	65.7 (59.5-71.3)	74.8 (64.3-111.1)
Combined LD50, mg/kg (95% C.L.)	68.9 (63.7-75.3)	
Highest Non-Lethal Dose, mg/kg	45	0
Lowest Lethal Dose, gm/kg (#dead/#tested)	60 (3/10)	45 (1/10)
Total Deaths/# Given Lethal Doses	19/30	14/40
Day of Deaths Day 1:	19/19	14/14
All deaths occurred within 10 min of dosing. There were 4 and 0 surviving males in the 70 and 85 mg/kg groups, respectively and 4 and 4 surviving females in the 70 and 85 mg/kg group, respectively.		
dl-ASL-8052		
Doses Tested, mg/kg	45, 60, 70, 85	
LD50, mg/kg (95% C.L.)	56.7 (50.2-62.1)	61.1 (52.4-69.1)
Combined LD50, mg/kg (95% C.L.)	58.3 (53.9-62.2)	
Highest Non-Lethal Dose, mg/kg	0	0
Lowest Lethal Dose, gm/kg (#dead/#tested)	45 (1/10)	45 (1/10)
Total Deaths/# Given Lethal Doses	26/40	22/40
Day of Deaths Day 1:	26/26	22/22
All deaths occurred within 10 min of dosing. There were no surviving males in either the 70 and 85 mg/kg groups. there were 3 and 1 surviving females in the 70 and 85 mg/kg groups, respectively.		

Separate groups of 10 rats (CD)/sex/dose received a single dose of either l-ASL-8052 (lot No. 907-40) or dl-ASL-8052 (lot No. 907-37) intravenously (tail vein). Mortality, signs of toxicity, and body weight were recorded over a 14 day observation period. ASL-8052 was dissolved in appropriate volumes of 0.9% Sodium Chloride, USP. Each dose was administered in a volume of 10 ml/kg at a rate of 2.0 ml/min (controlled by a syringe pump).

Signs of Toxicity were seen at all doses and were dose-related. The most common signs seen with both compounds included prostration, ataxia and dyspnea. Sedation was relatively common with l-ASL-8053 but not with the dl-form. Tremors and clonic Convulsions were only occasionally seen with both compounds. All toxic signs disappearance within 10 minutes. There was no notable drug-related difference in weight gains of survivors.

NOTE: The Sponsor did not indicate signs of toxicity in animals which died. The most common signs at the two highest doses were prostration and dyspnea.

Summary: In rats, the acute i.v. toxicity of l-ASL-8052 and dl-ASL-8052 were similar but the levo-isomer tended to be slightly less toxic (combined LD_{50}: 68.9 mg/kg vs 58.3 mg/kg) and caused a higher incidence of sedation than the dl-isomer. Other signs of toxicity included prostration, ataxia, dyspnea, tremors, and clonic convulsions. The highest non-lethal dose was less than 45 mg/kg (lowest dose tested). All deaths occurred within 10 minutes of injection and in survivors all signs of toxicity disappearance within 10 minutes.

(7) Dog: Two beagle dogs/sex received increasing i.v. doses of levo-ASL-8052 or dl-ASL-8052 ranging between 30 mg/kg to 240 mg/kg. At least 24 hrs was allowed between doses. Additional dogs were assigned to replace animals that died during study. Surviving dogs were observed for 14 days after the last dose and were then necropsied.

levo- and dl-ASL-8052 were dissolved in appropriate volume of 0.9% Sodium Chloride Injection USP to make concentrations of 6, 12, 24, 36 and 48 mg/ml. Each concentration was infused (syringe pump) i.v. (cephalic vein) at a flow rate of 5 ml/kg for 1 hour deleriving, therefore, total doses of 30, 60, 120, 180 and 24 mg/kg.

Except for salivation in 1/4 dogs at 120 mg/kg of dl-ASL-8052, no effects were observed with either,levo-ASL-8052 of dl-ASL-8052 at up to 120 mg/kg. At 180 mg/kg (3000 mcg/kg/min) dogs receiving levo-ASL-8052 exhibited tremors (2/4), salivation (2/4) and ataxia (2/4) while those receiving dl-ASL-8052 additionally exhibited hyperpnea, sedation and mortality (2/4). At 240 mg/kg (4000 mcg/kg/min) produced severe toxic effects consisting of sedation, tremors, ataxia, prostration, urination and tonic convulsions. These resctions were so severe that the infusion was stopped at 31 to 43 min in 3 of the 4 levo-ASL-8052 treated dogs and at 16 to 32 min in 3 of the 4 dl-ASL-8052 treated dogs. The levo-ASL-8052 treated dogs recovered within 5 minutes of stopping the infusion but all 3 of the dl-ASL-8052 treated dogs died (within minutes of stopping the infusion). No gross abnormalities were observed at necropsy.