CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER: 20-533/S-002

PHARMACOLOGY REVIEW

REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA
Division of Anesthetic, Critical Care & Addiction Drug Products

NDA: 20-533 Supplement: 002 IND: 31,121

Submission: NDA Supplement Dated: Sept. 24, 1998
Received by CDR: Sept. 28, 1998
Received by Reviewers: Oct. 1, 1998
Review Completed: May 18, 1999,
Reviewer: M. Anwar Goheer, Ph.D.

Sponsor: Astra Pharmaceutical, L.P.
725 Chesterbrook Boulevard
Wayne, PA 19087-5677

Information to be conveyed to the sponsor: Yes

Drug: Ropivacaine HCl monohydrate, LEA-103

Category: Local Anesthetics

Indications: Surgical Anesthesia: epidural block for surgery including cesarean section, major nerve block; local infiltration.
Acute Pain Management: epidural continuous infusion or intermittent bolus e.g., postoperative or labor; local infiltration.

Related NDAs: NDA 16-964 Bupivacaine (Marcaine, Sensorcaine)
NDA 17-751 Etidocaine (Duranest)

Names:

Chemical name: (S)-(-)-1-Propyl-2', 6'-pipecoloxylidide HCl H_20

IUPAC name: (S)-(-)-1-Propyl-piperidine-2-carboxylic acid (2, 6-dimethyl-phenyl)-amide hydrochloride monohydrate

Generic name: Ropivacaine hydrochloride monohydrate

CAS number: 132112-35-7

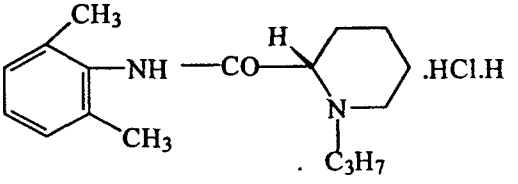
Trade name: NAROPIN™ Laboratory code name: LEA-103

Physical and Chemical characteristics:

Appearance: A white, crystalline powder

Melting range: 269.5 - 270.6°C
Solubility: The solubility in water at 25°C is 0.164 mol/L (53.8 mg/ml)

Structural formula:



Ropivacaine hydrochloride monohydrate is structurally related to the chemical group of amino-amides in present clinical use, e.g. bupivacaine and mepivacaine.

Molecular weight: 328.89

Molecular formula: $C_{17}H_{26}N_2O \times HCl \times H_2O$

Ionization constant: The pK_a value is 8.07 as determined by potentiometric titration at 25°C in (Dissociation constant) 0.1 M potassium chloride solution.

Optical rotation: $[\alpha]_D^{20} = -7.28°$ (C=2, H2O)

Manufacturer: Astra Production Chemicals AB, Strangnasvagen 20
S-151 85 Sodertalje, Sweden

Preclinical Studies:

Previously Reviewed Submissions: (a). Misoon Chun reviewed the following studies on April 18, 1988.

I. PHARMACOLOGY

(1) Primary Effects (Local Anesthetic Action)

1. Conduction block - in vitro and in Vivo studies
2. Infiltration anesthesia
3. Topical anesthesia
4. Action on smooth muscle
5. Behavioral effects
6. Action on different transmitter receptors
7. Monoamine oxidase inhibitory effect

(2) Secondary Effects

1. Effects on the cardiovascular system
a) In vivo studies
Intravenous administration -
anesthetized pig-
Conscious rat - with and without adrenaline
Conscious dog
Intracoronary administration - anesthetized pig
Effects of Pretreatment with atropine - conscious rat

b) In vitro studies

Effects on the isolated heart - rat
Effects on sodium and calcium conductance in the papillary muscle - guinea-pig
Effects on conductance in Purkinje fibers ventricular muscle cells - rabbit
Effects on the portal vein - rat

2. Effects on the central nervous system

Intravenous administration
Conscious rat (with and without adrenaline)
Conscious dog
Effects of diazepam and thiopentone on LEA 103 induced CNS toxicity-conscious rat

(3) Interactions

Anticholinergic - Atropine
Tranquilizers - Diazepam
Analgesics - Morphine, Fentanyl
General Anesthetic Agents - Thiopentone, Enflurane
Muscle Relaxants – Pancuronium, Suxamethonium

II. ACUTE TOXICITY

III. SUBCHRONIC TOXICITY STUDIES

1. Dose range-finding study of LEA 103 given subcutaneously to rats for 2 weeks
2. General toxicity of LEA 103 given subcutaneously to rats for one month
3. A dose range finding and MTD study of LEA 103 given subcutaneously to dogs for 3 days
4. General toxicity of LEA 103 given subcutaneously to dogs for one month

IV. SPECIAL TOXICITY STUDIES

1. Hemolysis and protein flocculation in human blood of LEA 103 studied in vitro
2. Vaso- and tissue irritation study in dogs of LEA 103 given intravenously and

12 page(s) have been removed because it contains trade secret and/or confidential information that is not disclosable.

Note - Portions of this review were excerpted directly from the sponsor's submission. Studies reviewed previously are summarized in the evaluation sections. The code names cited in studies for ropivacaine (Naropin) and its salts are LEA101, LEA103 and AL381.

Studies Reviewed

A. Pharmacology Studies

(1). Report No. 802-550-LF-0418-0I (F58): Evaluation of the Local Anesthetic Effects of Metabolites of Ropivacaine in the Guinea Pig. Vol. 3/page 106.

Test compounds:

Number	Generic name	Compound code	Batch no.	Supplier
1	S-PPX	LEA105	QA 283/21	Astra Pain Control
2	(R,S)-2-OH-Methyl-ropivacaine	LEA166	QA 859/13	Astra
3	(R,S)-4-OH-Ropivacine	LEA144	QA 860/09 or 660/07	Astra
4	(R,S)3-OH-PPX	LEA140	QA 779/15	Astra
5	(R,S)-3-OH-Ropivacaine	LEA145	QA 859/09	Astra
6	Ropivacine HCl monohydrate	LEA103	338/02	Astra

Species: Guinea pig.
Strain: white Dunkin-Hartley
Sex: Male (28-48 days old, 300-500 g body weight)
Supplier: [redacted]
Dosage and dose groups

Anesthesia	Concentration	Vol. of administration	Number/ group	Number of groups
Infiltration	1.25 – 10 mg/ml	0.25 ml	6	12
Sciatic nerve block	20 – 22 mg/ml	0.20 ml	6	4

Study dates: April 12, 1995 to June 29, 1996
Experimental procedures: Infiltration anesthesia after intracutaneous injection was performed

according to Bulbring and Wadja (J Pharmacol Exp Ther 1945; 85:78-84). Sciatic nerve block was performed by injecting the compounds in the hind leg of each animal according to Shackell (Anesth Analg 1935; 14:20-22). Mann-Whitney U test, sigma Stat version 2 was used to do statistical analysis.

Results

Effects of the metabolites of ropivacaine in the intracutaneous wheal test in the guinea pig.

Compound	conc mg/ml	% anaesthesia at different times after injection (min)						
		2	5	10	15	20	30	120
ropivacaine	5	100	100	100	99.1	99.1	99.1	87.1
s.e.m		0	0	0	1.6	1.6	1.6	7.6
S-PPX	10	86.1*	94.4	94.4	91.7	77.8*	61.2*	0
s.e.m		2.8	3.5	3.5	5.6	12.6	12.7	0
R,S-3-OH-ropivacain	10	97.2	100	94.4	61.2*	33.3*	16.8*	0
s.e.m		2.8	0	3.5	12.7	12.2	10.6	0

*p<0.05 versus 5 mg/ml ropivacaine

Intracutaneous wheal test in the guinea pig.

Compound	conc mg/ml	% anaesthesia at different times after injection (min)						
		2	5	10	15	20	30	120
ropivacaine	2.5	97.2	97.2	100	100	100	95.8	56.9
s.e.m		1.9	1.9	0	0	0	2.2	9.3
S-PPX	2.5	80.6	66.9*	41.8*	5.5*	0		
s.e.m		7.9	4.3	9.4	5.5	0		
R,S-3-OH-ropivacain	2.5	22.3*	5.7*	0*				
s.e.m		3.4	3.6	0				
ropivacaine	1.25	97.2	97.2	97.2	94.5	88.9	80.6	0
s.e.m		2.8	2.8	2.8	5.5	5.5	2.7	0

*p<0.05 versus 2.5 mg/ml ropivacaine

Effects of the metabolites of ropivacaine in the intracutaneous wheal test in the guinea pig.

Compound	conc mg/ml	% anaesthesia at different times after injection (min)						
		2	5	10	15	20	30	120
ropivacaine	5	100	100	100	99.1	99.1	99.1	87.1
s.e.m		0	0	0	1.6	1.6	1.6	7.6
S-PPX	5	97.2	91.6	77.9*	44.6*	36.2*	0*	
s.e.m		2.78	3.73	5.49	11.1	7.95	0	
R,S-3-OH-PPX	5	11.2*	2.8*	0*				
s.e.m		8.2	2.8	0				
R,S-4-OH-ropivacaine	5.17	50.2*	25.2*	2.8*	0*			
s.e.m		8.7	11.2	2.8	0			
R,S-3-OH-ropivacaine	5	91.7	91.7	61.3*	22.3*	13.6*	0*	
s.e.m		3.7	5.6	14.6	11.9	9.0	0	
R,S-2-OH-methyl-ropi	5	80.7*	63.9*	22.2*	5.5*	0*		
s.e.m		6.6	10.9	11.1	5.5	0		

*p<0.05 versus 5 mg/ml ropivacaine

Sciatic nerve block in the guinea pig

Compound	conc. mg/ml	Motor block minutes	
		Onset	Duration
S-PPX	22		
Median		0	0
Range		0	0
Quartile 1		0	0
R,S-4-OH-ropivacaine	20		
Median		0	0
Range		0	0
Quartile 1		0	0
R,S-3-OH-ropivacaine	20		
Median		5	51.5
Range		2	16
Quartile 1		4	43
ropivacaine	20		
Median		4	80
Range		2	20
Quartile 1		3	80

Sciatic nerve block in the guinea-pig

Compound	conc. mg/ml	Sensory block minutes	
		Onset	Duration
S-PPX	22		
Median		3	10
Range		0	1
Quartile 1		3	10
R,S-4-OH-ropivacaine	20		
Median		2.5	5
Range		5	12
Quartile 1		0	0
R,S-3-OH-ropivacaine	20		
Median		5	46
Range		1	26
Quartile 1		5	40
ropivacaine	20		
Median		4	70
Range		2	19
Quartile 1		3	70

All metabolites of ropivacaine except 3-OH-PPX produced less and shorter duration of anesthesia compared to parent compound.

Number	Generic name	Compound code	Batch no.	Supplier
1	S-PPX	LEA105	QA 283/21	Astra Pain Control
2	(R,S)-2-OH-Methyl-ropivacaine	LEA166	QA 859/13	Astra
3	(R,S)-4-OH-Ropivacine	LEA144	QB660/07	Astra
4	(R,S)-3-OH-Ropivacaine	LEA145	QA 859/09	Astra

pH was adjusted with 0.2M NaOH to ~6.

Species: Mice.

Strain: NMRI
Sex: Male (17-23 g body weight)
Supplier: [redacted]
Dosage

LEA105 (S-PPX)	4.7, 9.3, 18.6, 27.9 and 37.2 mg/kg (20, 40, 80, 120 and 160 μmol/kg)
LEA144 (R, S-4-OH-ropivacaine)	11.6, 23.2 and 46.5 mg/kg (40, 80, and 160μmol/kg)
LEA145 (R, S-OH-ropivacaine)	5.8, 11.6 and 23.2 mg/kg (20, 40 and 80 μmol/kg)
LEA166 (R, S-2-OH methyl-ropivacaine)	11.6, 23.2, 29.0, and 46.5 mg/kg (40, 80, 100,and 160 μmol/kg)
Physiological saline	10 ml/kg

Dose volume and dose groups: 10 ml/kg and ~5 animals/group
Study dates: October 3, 1996 – Feb. 2, 1998
Experimental procedures: Rapid (<5 second) intravenous injection in to a tail vein
Data Analysis: No statistical analysis was performed.

Results:

Control animals: Lacrimation (4/19), mydriasis (2/19).
S-PPX (LEA105) treated animals: Lacrimation (not dose dependent), twitches (2/5) and convulsion (1/5) at high dose (120 μmol/kg). Vocalization and touch response (2/5) only at 80 μmol/kg group.
R, S-4-OH-ropivacaine (LEA144) animals: Increased spontaneous activity and touch response at 40μmol/kg. All three animals given 160 μmol/kg died.
R, S-3-OH-ropivacaine (LEA145): Increased touch response and lacrimation at 40 μmol/kg. 80 μmol/kg killed 2/4 animals immediately after injection.
R, S-2-OH-methyl ropivacaine: Decreased grip strength and lacrimation (2/4 animals) at 40 μmol/kg. No deaths were observed.

Doses of ropivacaine, metabolites of ropivacaine and bupivacaine causing convulsions and deaths in mice are shown below.

Compound	Convulsions (μmol/kg)	Mortality (μmol/kg)
S-PPX	120	160
R,S-3-OH-ropivacaine	n.d.	80
R,S-4-OH-ropivacaine	80	160
R,S-2-OH-methyl-ropivacaine	160	n.t.
ropivacaine	23	39
bupivacaine	23	28

n.d. = not detected
n.t. = not tested

(3). Report No. 802-550-LF-0296-01 (F60): Comparison of Cardiac Effects of Ropivacaine and Bupivacaine Enantiomers. Vol. 3/page 147.

Project no.: 802-550
Study site: [redacted]
Experimental period: April 8, 1994 – May 17, 1994
Test and reference compounds:

Compound	Code number
S-ropivacaine	AII
R-ropivacaine	AIII
S-bupivacaine	AIV
R-bupivacaine	AV
S/R-bupivacaine	AVI

Animals: Albino rabbits, ~ 6 weeks old, ~ 1kg body weight.
Supplier: [redacted]
Doses: 1, 3 and 10 μM
Experimental procedure: Resected hearts were perfused within 15 to 45 seconds, mounted and allowed to recover for 30 to 60 minutes. The experiment was started approximately one hour after the surgery. All measurements were done automatically with computer. Minimum acceptance criteria and normal values for some of the measured parameters are give below.

Parameter	Accepted value	Normal value
Automaticity cycle length	< 2000 msec	> 15000 msec
Escape cycle length	< 1000 msec	> 15000 msec
Preload for maximum developed pressure	<15 mm Hg	5-8 mm Hg
Developed pressure	> 30 mm Hg	~ 90 mm Hg
Threshold stimulation current	< 300 μA	~ 100 μA
Coronary perfusion	>10 ml/min	~ 17 ml/min
Cardiac activation time	< 50 msec	~ 40 msec

All five compounds slowed conduction in a use-dependent fashion, which is a indicative of block of sodium channels in the heart. Both ropivacaine enantiomers appeared to be less arrhythmogenic than S-bupivacaine and S/R-bupivacaine. S-ropivacaine appeared to be less negative inotropic.

B. Toxicology

(1). Report No.802-51 T3320 (QI): Single Dose Toxicity of a Combination of Ropivacaine and Fentanyl in Rats after Subcutaneous Administration. Vol. 4/page 47

Test compound	Code	Molar mass	Batch	Purity (%)
Ropivacaine HCl monohydrate	LEA103	328.9	200193	99.9
Fentanyl citrate		528.6	21772	99.7

Supplier: Astra Pain Control AB, Sweden
Animals: Wistar male and female rats, 8 weeks old, 140 – 350 g body weight,
Supplier: [redacted]
Route: Subcutaneous
Sponsor: Astra Pain Control AB, Sweden.

Number & Sex	Test Compound (s)	Dose Levels (μMol/kg)	Dose Levels (mg/kg)
2M + 2F	Ropivacaine	100	31
5M + 5F	Ropivacaine	200	62
5M + 5F	Ropivacaine	280	87
5M + 5F	Ropivacaine	390	120
5M	Fentanyl	0.2	0.067
5 M + 5F	Fentanyl	0.5	0.17
5M	Fentanyl	1.9	0.64
5M	Fentanyl	3.0	1.0
5M + 5F	Fentanyl	4.2	1.4
2M + 2F	Fentanyl	10	3.4
2M + 5F	Fentanyl	15	5.0
5F	Fentanyl	20	6.7
2F	Fentanyl	25	8.4
5M	Ropivacaine	200	62
	Fentanyl	0.5	0.17
5M	Ropivacaine	200	62
	Fentanyl	0.25	0.084
5M	Ropivacaine	100	31
	Fentanyl	0.5	0.17
5M	Ropivacaine	100	31
	Fentanyl	0.25	0.084

Number & Sex	Test Compound (s)	Dose Levels (μMol/kg)	Dose Levels (mg/kg)
5F	Ropivacaine	200	62
	Fentanyl	5.0	1.7
5F	Ropivacaine	200	62
	Fentanyl	2.5	0.84
5F	Ropivacaine	100	31
	Fentanyl	5.0	1.7
2F	Ropivacaine	100	31
	Fentanyl	2.5	0.84
5M	Ropivacaine	60	19
	Fentanyl	0.15	0.05
5M	Ropivacaine	30	9.3
	Fentanyl	0.075	0.025
2F	Ropivacaine	60	19
	Fentanyl	1.5	0.50
5F	Ropivacaine	30	9.3
	Fentanyl	0.75	0.25
5F	Ropivacaine	30	9.3
	Fentanyl	0.25	0.084

GLP/ QA statements: Yes
Study duration: Sept. 27, 1994 – Nov. 3, 1994
Clinical observations:
Clinical signs and mortality: Once during the intervals, 0.0 –0.5, 0.5 – 1.0, 1.0 – 3.0, 3 – 6 and 6 – 24 hours and then daily for 14 days after dosing.
Body weight: Before dosing and then on days 1, 2, 3, 4, 7, 10, and 14
Food consumption: Free access but not recorded.
Pathology: Necropsy included abdominal, thoracic and cranial cavities, with their respective organs.

Results:

Clinical signs:

Ropivacaine alone – Reduced motor activity and abdominal respiration at all doses Piloerection, tremor, ataxia, twitching and convulsions at higher doses

Fentanyl alone – Reduced motor activity, prostration, absent blinking reflex, cyanosis, irregular respiration, piloerection, salivation, ataxia, increased muscular tonus and convulsion in a dose-dependent manner.

Ropivacaine plus fentanyl – Similar clinical signs as noted above but very much

pronounced at lower doses.

Mortality ratios: The minimum lethal and maximum non-lethal doses

Compound	Minimum lethal dose (μmol/kg)		Maximum non lethal dose (μmol/kg)	
	Males	Females	Males	Females
Ropiovacaine	280	200	200	100
Fentanyl	0.5	20	0.2	15
Ropivacaine + fentanyl	60 + 0.15	30 + 0.75	30 + 0.075	30 + 0.25

Body weight: No effect

Gross pathology:

Ropivacaine: 390 μmol/kg - Lung edema (3/8)

280 μmol/kg – Grayish-white foci of the lungs (2/10)

Fentanyl: - Foci in the glandular stomach in high dosed animals (3.0 & 4.2 μmol/kg)

Ropivacaine + fentanyl: Skin and lung lesions

Microscopic pathology:

Ropivacaine: 280 and 390 μmol/kg – Congestion and edema of the lungs, focal granulomas and perivascular cuffing of the lungs, ulcerative dermatitis at the injection sites

Fentanyl: Lung and skin changes similar to ropivacaine treated animals.

Ropivacaine + fentanyl: Same type of lung and skin changes as mentioned above.

A synergistic toxicity was seen when ropivacaine and fentanyl were given together compared to when they were given alone.

(2). Report No.802-51 T3314 (Q2): Dose Finding Study of a Fixed Combination of Ropivacaine and Fentanyl Given Subcutaneously to Dogs for Up to 5 Days. Vol. 4/page 204.

Test compound	Code	Molar mass	Batch	Purity (%)
Ropivacaine HCl monohydrate	LEA103	328.9	200193	[redacted]
Fentanyl citrate		528.6	21772	[redacted]

Animals: Beagle dog, 7.5 – 14.8 months old, 9.6 – 15.3 kg body weight

Route: Subcutaneously in the neck region.

GLP/QA statements: Yes

Clinical signs, body weight, food consumption, rectal temperature, electrocardiography, hematology, blood chemistry, gross and microscopic pathology were performed.

Results: The maximum tolerated doses of ropivacaine and fentanyl in dogs when given in combination once daily for five days were 15.0 + 0.068 µmol/kg, respectively. This experiment was done to establish a suitable high dose level in one-month toxicity study.

Test compound	Code	Molar mass	Batch	Purity (%)
Ropivacaine HCl monohydrate	LEA103	328.9	200/94	
Fentanyl citrate	LEF244	528.6	21772	

Animals: Beagle dog, 8.5 months old, 9.3 – 12.3 kg body weight
Supplier:
Route: Intravenous for 3 hours.
GLP/QA statements: Yes

Results:

Clinical signs: Drowsiness and tremor and seizures at the highest dose tested (Ropivacaine 35 + fentanyl 0.065 µmol/kg).
Body weight, food consumption and rectal temperature: No effect
Electrocardiography: Slight reduction of heart rate in most animals.
Gross and microscopic pathology: No findings in brain, heart, liver, gallbladder, kidneys and intravenous injection site.

An intravenous infusion of 1.8 ml/kg.hr for 3 hours with a combination of 6.4 µmol/ml ropivacaine and 0.012 µmol/ml fentanyl was considered as a maximum tolerated dose in dogs

Test compound	Code	Molar mass	Batch	Purity (%)
Ropivacaine HCl monohydrate	LEA103	328.9	200193	
Fentanyl citrate		528.6	21772	

Animals: Beagle dog, 10 – 16 months old, 11.3 – 16.1 kg body weight
Supplier: [redacted]

Group #	Group Name	Compound	Concentration µmol/ml	Concentration mg/ml	Dose µmol/dog	Dose mg/dog
1	Control	Saline				
2	Ropivacaine	Ropivacaine HCl	19	6.0	58	18
3	Fentanyl	Fentanyl	0.045	0.015	0.13	0.045
4	Ropi + Fent. Low	Ropivacaine HCl + Fentanyl	6.4 + 0.015	2.0 + 0.005	19.0 + 0.044	6.0 + 0.015
5	Ropi + Fent. High	Ropivacaine HCl + Fentanyl	19 + 0.044	6.0 + 0.015	58 + 0.13	18 + 0.045

Dose volume: 3 ml.
Route: Epidural injection.

Clinical observation: General and neurological signs (locomotive faculty [paralysis, paresis, atactic or normal movement], reflexes [anal reflex and pedal reflex], postural reactions [extensor postural thrust and proprioception], muscular tonus [atonic, hypotonic, normal or spastic] and pain sensation [superficial and deep] of the hind legs. Muscular tonus was investigated in the tail, anal sphincter and hind legs. The spread of the anesthesia was investigated using the panniculus reflex.
Body weight, food consumption, rectal temperature, plasma concentration of the test compounds, gross and microscopic pathology were performed
One male and one female in each group were necropsied 5 days after dosing. Remaining animals (1/sex/group) were necropsied 14 days after dosing.
GLP/QA statements: Yes

Results:

Body weight, food consumption and rectal temperature (on days 5 and 14): No effect
Gross Pathology: No gross lesions were noted in the spinal cord.

Microscopic pathology:

NUMBER OF ANIMALS WITH MICROSCOPIC FINDINGS BY ORGAN/GROUP/SEX
STATUS AT NECROPSY: K0

DOSE GROUP:	1		2		3		4		5	
SEX:	M	F	M	F	M	F	M	F	M	F
NO.ANIMALS:	2	2	2	2	2	2	2	2	2	2
SPINAL CORD, SACRAL :	2	2	2	2	2	2	2	2	2	2
- EPIDURAL VASCULITIS :	-	-	-	-	-	-	-	-	1	-
SPINAL CORD, LUMBAR :	2	2	2	2	2	2	2	2	2	2
- EPIDURAL DYSCALCIFIC:	-	-	-	-	-	-	-	1	-	-
- EPIDURAL INFLAM. :	-	-	1	-	-	-	-	-	-	-

No dose-dependent pathological changes were observed.

Test compound	Code	Molar mass	Batch	Purity (%)
Ropivacaine HCl monohydrate	LEA103	328.9	201/93	
Fentanyl citrate	LEF244	528.6	21772	

Control: Saline (0.9% NaCl)
Supplier: Astra Pain Control AB, Sodertalje, Sweden
Animals: Beagle dogs, 4 – 5 months old, 6 – 10 kg body weight
Supplier:

Blood samples: About 5 ml of the whole blood from cephalic vein.

Study design:

Group number	Group designation	Dose concentration R/B µmol/ml	R/B mg/ml	F µmol/ml	F µg/ml	Dose volume ml/kg/h	Dose level R/B µmol/kg.h	R/B mg/kg.h	F µmol/kg.h	F µg/kg.h	Number of animals Males	Females
1	Control	0	0	0	0	0.083	0	0	0	0	3	3
2	Low dose Ropivacaine + Fentanyl	6.4	2.0	0.012	4.0	0.083	0.53	0.17	0.0010	0.33	3	3
3	High dose Ropivacaine + Fentanyl	13	4.0	0.024	8.0	0.083	1.1	0.33	0.0020	0.66	3	3
4	Bupivacaine + Fentanyl	13	4.2	0.024	8.0	0.083	1.1	0.35	0.0020	0.66	3	3
5	Fentanyl	0	0	0.024	8.0	0.083	0	0	0.0020	0.66	3	3

R/B : ropivacaine/bupivacaine.

F : fentanyl.

Animals in-groups 3, 4 and 5 were dosed with 50% of the final dose on day 1, 75% of the final dose on day 2, and full final dose on day 3.

Method of administration: Continuous epidural infusion, 24 hours per day, using a catheter implanted into the epidural space between the last lumbar and the first sacral vertebrae.

Flow rate during treatment period: 0.083 ml/kg/hour

Duration of treatment: 29 or 30 days (groups 3-5 were treated for 27 to 28 days at the high dose level).

Study site: [redacted]

GLP/QA statements: Yes

Study sponsor: Astra AB, Safety Assessment, S-151 85 Sodertalje, Sweden

Observations:

Morbidity/mortality: Twice daily

General Observations: Daily for the first 4 –6 days of infusion, then on days 7, 10, 14, 17, 21, 24, and immediately before the end of treatment

Body weight: Weekly

Food Consumption: Daily

Rectal temperature: Daily for first 4 days, on day 7 and then twice a week

Ophthalmology: Pretest and before termination.

Cardiovascular examinations: Once pretest, once during testing and once during week 4.

Clinical pathology: Hematology, blood clinical chemistry and urine analysis were done.

Pathology: Necropsy, organ weights and histopathology were performed.

Results:

Clinical signs: Neurological signs.

Ropivacaine/bupivacaine + fentanyl – Stiffness of the hindquarters, paresis or paralysis, absence of patellar, pedal and panniculus reflexes and superficial pain sensation. Loss of anal reflex was also observed.

Fentanyl – Transient bilateral stiffness of the hindquarters on day 2 (1 animal), firm abdomen on day 17 of the same animal

Mortality: None

Body weight: Groups 2 and 3 – No effect

Group 4 – (15%↓ M, & 7↓%F)

Group 5 – No effect

Food consumption: No significant differences

Rectal temperature: No consistent differences

Ophthalmology: No effect

Cardiovascular examination:

Heart rate – No difference

Rhythm and cardiac conduction – No effect

Hematology: No differences

Blood clinical chemistry: No effect

Urine analysis: No differences

Organ weights: No consistent differences

Macroscopic findings:

	Animal no.				
Finding	Group 1	Group 2	Group 3	Group 4	Group 5
Injection site					
Dark areas on surface of s.c	453		463 - 464 - 466 467 - 468	469 - 471 - 472	478
Swelling				470 - 473	
Implantation site					
Mass	455		465		
Swelling			463		
Location of catheter tip					
Subdura (lumbar)	452 - 456			474	
In spinal cord			466		
Subcutaneously (lumbar)		458			475
Implantation site (mass)			465		

Microscopic findings: No systemic changes related to treatment.

Findings (grade)\group number	Males 1	2	3	4	5	Females 1	2	3	4	5
Fibrosis around catheter										
Slight	2	1	0	3	0	2	0	1	2	2
Moderate	0	1	1	0	2	0	3	2	1	1
Epidural inflammation										
Minimal	1	0	0	0	0	1	0	1	1	0
Slight	1	1	0	2	1	1	1	1	1	2
Moderate	0	1	1	1	1	1	2	1	0	1
Superficial dural inflammation										
Minimal	0	0	0	0	0	1	0	0	0	0
Slight	1	1	0	0	0	1	1	0	1	1
Moderate	0	0	2	2	1	0	1	1	2	1
Marked	0	0	0	1	0	0	0	1	0	0
Fibrosis of dura										
Minimal	0	0	0	0	0	0	1	1	0	0
Slight	0	0	0	0	0	0	0	1	0	0
Moderate	0	0	0	1	0	0	0	1	1	1
Marked	0	0	0	0	0	0	0	0	1	0

Toxicokinetics: Mean plasma concentrations (Males + Females) of the test compounds.

There were no consistent differences between males and females. The Css of fentanyl showed an increase with time in all groups.

Treatment	Days	Css R/B (µmol/L)	Css F (nmol/L)	CL R/B (mL/min.kg)	CL F (mL/min.kg)
R + F (low)	Day 1, mean (R + F)	0.273±0.070	0.153±0.013	35.2±13.7	109.4±8.7
	Day 30/31, mean (R + F)	0.351±0.079	0.221±0.027	26.7±8.1	76.1±8.0
R + F (high)	Day 3 to 30/31, overall mean (R)	0.812±0.232		24.7±7.0	
	Day 3, mean (F)		0.306±0.055		112.9±25.5
	Day 30/31, mean (F)		0.469±0.120		74.8±17.6
B + F	Day 3, mean (B + F)	1.299±0.234	0.350±0.102	14.5±2.7	100.6±22.1
	Day 30/31, mean (B + F)	0.871±0.290	0.426±0.064	23.0±7.7	79.8±13.4
F	Day 3, mean (F)	-	0.324±0.074	-	107.3±23.2
	Day 30/31, mean (F)	-	0.488±0.202	-	94.2±80.4

R = ropivacaine B = bupivacaine F = fentanyl

Test compound: LEA105 (primary metabolite of ropivacaine)
Molecular weight – 232.3, batch 103/96, ——— supplier – Astra.
Control substance: Saline
Animal: Male and female Sprague Dawley rats, 7 – 8 weeks old, 204 – 343 g body weight.
Food and water: ad libitum.

Route: Subcutaneous administration
Dose volume: 10 ml/kg, single dose
Dose groups:

Dose Group	LEA105 Treatment		Number of Males in Group (Animal Numbers)	Number of Females in Group (Animal Numbers)
	µmol kg^{-1}	mg kg^{-1}		
1	0.0	0.0	5 (1,2,3,4,5)	5 (6,7,8,9,10)
2	500	116	0	5 (46,47,48,49,50)
3	1000	232	5 (21,22,23,24,25)	5 (26,27,28,29,30)
4	1500	348	0	5 (66,67,68,69,70)
5	2000	465	5 (11,12,13,14,15)	4 (16,17,18,19)
6	2500	581	5 (31,32,33,34,35)	0
7	4000	929	5 (51,52,53,54,55)	0

Observation period: 14 days after dosing.
Sponsor: Astra Pain Control AB, Sweden.
GLP/QA statements: Yes

Results:

Clinical observations: Reduced respiratory and motor systems in females at 500 µmol/kg and in males at 1000 µmol/kg. Dark and thickened skin at the injection sites in a dose-dependent manner.

Mortalities:

µmol-kg^{-1} LEA105	Number of Mortalities	
	M	F
0	0/5	0/5
500	-	0/5
1000	0/5	0/5
1500	-	0/5
2000	0/5	4/4
2500	0/5	-
4000	1/5	-

Body weight: Dose-related decrease in body weight gain in male rats only.

Dose Group	Dose Level (µmol·kg⁻¹)	Day 1 Group Mean Bodyweight (g)	Gain (g)						
			Day 1-2	Day 2-3	Day 3-4	Day 4-5	Day 5-8	Day 8-15	Total Day 1-15
1	0	327.0	-0.2 (5)	+8.6 (5)	+5.0 (5)	+5.2 (5)	+15.2 (5)	+35.2 (5)	+69.0
3	1000	328.0	+0.6 (5)	+3.4 (5)	+0.4 (5)	+4.8 (5)	+11.4 (5)	+29.2 (5)	+49.8
5	2000	320.6	-9.0 (5)	+9.4 (5)	+0.2 (5)	-1.0 (5)	+10.8 (5)	+35.0 (5)	+45.4
6	2500	317.0	-17.6 (5)	+2.8 (5)	+2.6 (5)	-0.6 (5)	+13.0 (5)	+32.4 (5)	+32.6
7	4000	343.0	-22.3 (4)	-4.5 (4)	+6.5 (4)	+2.3 (4)	+15.3 (4)	+27.8 (4)	+25.1

(n) = number of animals

Females

Dose Group	Dose Level (µmol·kg⁻¹)	Day 1 Group Mean Bodyweight (g)	Gain (g)						
			Day 1-2	Day 2-3	Day 3-4	Day 4-5	Day 5-8	Day 8-15	Total Day 1-15
1	0	209.8	-1.2 (5)	+2.2 (5)	+4.2 (5)	+2.8 (5)	+7.2 (5)	+9.2 (5)	+20.4
2	500	208.6	+1.2 (5)	+3.4 (5)	+0.6 (5)	+1.0 (5)	+7.6 (5)	+8.4 (5)	+22.2
3	1000	203.8	+6.2 (5)	-3.8 (5)	+3.0 (5)	+1.4 (5)	+5.0 (5)	+14.4 (5)	+23.4
4	1500	212.4	+3.2 (5)	-3.4 (5)	+6.0 (5)	+1.0 (5)	+7.4 (5)	+10.6 (5)	+24.8

(n) = number of animals

Pathology: Necrosis and/or ulceration, inflammation and fibrosis at the injection sites in all treated animals. Minimal adrenal cortical vacuolation in 2/4 males dosed at 4000 µmol/kg and in 1/5 females at 1500 µmol/kg. Myocardial degeneration/fibrosis in ¼ males, hemopoiesis in 2/4 males at 4000 µmol/kg. Minimal inflammatory cell foci in the liver in 3/5 females at 1000 µmol/kg.

Species/strain: Rat/Sprague Dawley, 7 – 8 weeks old, and 165 – 327 g body weights.
Supplier: [redacted]
Study dates: 8 May 1997 – 27 May 1997
Compound: LEA105, batch no 103/96, supplied by Astra Pain Control, Sweden
Administration route: Intravenous, single administration, 0.42 – 0.63 ml/min via the lateral

tail vein
Observation period: 15 days
GLP/QA statements: Yes
Dose Groups:

Dose Group	LEA105 Treatment $\mu mol \cdot kg^{-1}$	LEA105 Treatment $mg \cdot kg^{-1}$	Number of Males in Group (Animal Numbers)	Number of Females in Group (Animal Numbers)
1	0.0	0.0	5 (1,2,3,4,5)	5 (6,7,8,9,10)
2	80	19	2 (11,12)	2 (16,17)
3	140	33	5 (21,22,23,24,25)	2 (26,27)
4	280	65	5 (31,32,33,34,35)	5 (36,37,38,39,40)
5	420	98	5 (41,42,43,44,45)	5 (46,47,48,49,50)
6	540	125	0	3 (61,63,64)
7	650	151	2 (51,52)	2 (56,57)

Results:

Clinical observation: 80 µmol/kg – Decrease/deep respiration and unsteadiness. Dose -dependent clinical effects were seen up to 4 hours post dose.
Mortalities:

$\mu mol \cdot kg^{-1}$ LEA105	Number of Mortalities M	F
0	0/5	0/5
80	0/2	0/2
140	0/5	0/2
280	0/5	0/5
420	2/5	0/5
540	-	3/3
650	2/2	2/2

Body weights:

Males

Dose Group	Dose Level ($\mu mol \cdot kg^{-1}$)	Day 1 Group Mean Bodyweights (g)	Gain (g) Day 1-2	Day 2-3	Day 3-4	Day 4-5	Day 5-8	Day 8-15	Total Day 1-15
1	0	281.0 (5)	-0.8 (5)	+10.8 (5)	+6.2 (5)	+5.4 (5)	+15.6 (5)	+41.0 (5)	+78.2 (5)
2	80	262.0 (2)	-3.5 (2)	+15.0 (2)	+6.0 (2)	+8.5 (2)	+18.5 (2)	+45.5 (2)	+90.0 (2)
3	140	286.2 (5)	+0.8 (5)	+7.4 (5)	+6.6 (5)	+6.6 (5)	+11.0 (5)	+40.4 (5)	+72.8 (5)
4	280	297.2 (5)	-1.2 (5)	+7.2 (5)	+8.6 (5)	+3.8 (5)	+16.2 (5)	+40.4 (5)	+75.6 (5)
5	420	296.6 (5)	-4.3 (3)	+4.7 (3)	+8.3 (3)	+4.0 (3)	+15.0 (3)	+49.0 (3)	+76.7 (3)

Females

Dose Group	Dose Level ($\mu mol \cdot kg^{-1}$)	Day 1 Group Mean Bodyweights (g)	Gain (g) Day 1-2	Day 2-3	Day 3-4	Day 4-5	Day 5-8	Day 8-15	Total Day 1-15
1	0	197.8 (5)	-3.6 (5)	+4.8 (5)	+2.6 (5)	+3.0 (5)	+5.0 (5)	+17.4 (5)	+29.2 (5)
2	80	192.0 (2)	+3.5 (2)	+1.0 (2)	-0.5 (2)	+0.5 (2)	+10.5 (2)	+23.5 (2)	+38.5 (2)
3	140	193.0 (2)	-2.0 (2)	+6.5 (2)	+3.5 (2)	+5.5 (2)	+7.0 (2)	+15.5 (2)	+36.0 (2)
4	280	187.2 (5)	+4.0 (5)	+3.2 (5)	+1.4 (5)	-4.6 (5)	+7.2 (5)	+17.2 (5)	+28.4 (5)
5	420	196.4 (5)	+3.4 (5)	+3.6 (5)	+0.6 (5)	+0.2 (5)	+7.6 (5)	+11.6 (5)	+27.0 (5)

(n) = number of animals

Pathology: Congestion, dermatitis and ulceration at the injection sites in one animal at 420 µmol/kg.

E [redacted] 1.

Species/breed: Beagle dogs, 7 – 13 kg body weight, 6 – 7 months old.
Supplier: [redacted]
Test articles: LEA103 – ropivacaine hydrochloride monohydrate [redacted]
batch no. 1202-2-1 [redacted], & 2146-1-1 [redacted]
mg/ml),
Supplier - Astra Pain Control, Sweden.
LEA131 – bupivacaine hydrochloride monohydrate. batch no – 2144-1-1 [redacted]
%,
Supplier - Astra Pain Control, Sweden.
Control: Saline for injection, Supplier – [redacted]
Study site: [redacted]
Study sponsor: Astra AB, Safety Assessment, S-151 85 Sodertalje, Sweden.
Study number: 265/509
Study dates: 6 June 1995 - 18 July 1995.
GLP/QA statements: Yes
Experimental procedure:

Group number	Treatment	Dose level (µmol/kg/h)	Dose level (mg/kg/h)	Dose volume (ml/kg/h)	Formulation conc. (µmol/ml)	Formulation conc. (mg/ml)	Number of animals Males	Number of animals Females
1	Control	0	0	0.083	0	0	3	3
2	LEA 103 - low dose	0.43	0.17	0.083	6.4	2.1	3	3
3	LEA 103 - high dose	1.1[1]	0.35[1]	0.083	13[1]	4.2[1]	3 + 1	3 + 1
4	LEA 131 - low dose	0.51	0.17	0.083	6.1	2.1	3	3
5	LEA 131 - high dose	1.0	0.35	0.083.	12	4.2	3	3

. Group 1 animals (control) received the control article (saline for injection).

Route: Epidural, continuous infusion (24 h/day) using a catheter implanted in to the epidural space (between the last lumbar and the first sacral vertebrae).
Examinations:
Morbidity/mortality: Twice daily.
Clinical observation: Daily
Body weight: Weekly
Food consumption: Daily
Blood samples: Pretest, on days 1, 3, 6, 12, 24, 48, and 72 h after start of treatment; once on days 10, 17 and 24 at a suitable time and then last day of treatment (via a cephalic vein).
Necropsy and histopathology: Full
Due to small number of animals (N=3), no statistical analysis was performed.

Results:

Clinical signs: Dose-dependent neurological sings (paresis, paralysis, absence of muscle tone, the patellar, pedal, panniculus or anal reflexes. Superficial and profound

pain sensations, negative extensor postural thrust and proprioception was also observed.) In each group the incidence of the neurological signs was higher in males than in females.

Mortality: Two group 3(high dose ropivacaine) animals showed reversible neurological signs and sacrificed on day 14.

Body weight: No effect

Food consumption: No differences.

Hematology: No differences.

Blood clinical chemistry: No differences in male no 63 and female no. 66.

Macroscopic findings: Dark red areas on the spinal cord at the catheter implantation.

Microscopic findings: Groups 2 & 3 (ropivacaine) – Minimal inflammation in the dura mater.
Group 4 (bupivacaine) – Inflammation extended into the arachnoid 4/6 animals)
Group 5 (bupivacaine) Inflammation extended in one female animal (1/6).
No changes in the nervous tissue in any group.

Pharmacokinetics data

Group	Tmax (h)	Cmax (µmol/l)	Css (µmol/l)	CL (ml/min/kg)	T1/2 (h)	AUC 0-24 h (µmol.h/l)	AUC 0-total (µmol.h/l)
2	104±89.4	0.52±0.11	0.39±0.06	23.9±3.8	0.92±0.4	225±48	282±47
3	145±215	0.91±0.2	0.76±0.16	25.3±5.9	2.51±1.6	428±74	515±107
4	188±166	0.58±0.1	0.42±0.05	22.4±2.5	0.59±0.1	245±24	290±25
5	84±67.5	1.20±0.4	0.88±0.24	22.0±5.6	0.76±0.2	484±98	531±123

The only significant difference between comparable doses of ropivacaine and bupivacaine was in the t1/2.

C. Pharmacokinetics

[redacted]

Animals: Male and female Sprague-Dawley rats (~2 months old, 170-250 g body weight)
Supplier [redacted]
Test compound: Ropivacaine (LEA103)
Source: Astra AB, Sodertalje, Sweden.
GLP/QA statements: Yes

Experimental Procedure: The satellite animals (58 males and 58 females) were divided into four dose groups for four sampling occasions as shown below. Ropivacaine gel was administered twice daily at 0, 20, 40 or 80 µmol/kg by a soft plastic tube inserted approximately 3 cm into the rectum.

Sampling occasion	Group	Animal	Dose µmol/kg	Sampling occasion	Group	Animal	Dose µmol/kg
Day 1	21	2M & 2F	Vehicle	Day 90	13	2M & 2F	Vehicle
	22	4M & 4F	20	(3 month)	14	4M & 4F	20
	23	4M & 4F	40		15	4M & 4F	40
	24	4M & 4F	80		16	4M & 4F	80
Day 14	17	4M & 4F	Vehicle	Day 181	9	2M & 2F	Vehicle
	18	4M & 4F	20	(6 month)	10	4M & 4F	20
	19	4M & 4F	40		11	4M & 4F	40
	20	4M & 4F	80		12	4M & 4F	80

Blood samples were taken at four different occasion during the study period; on days 1, 14 and 3 and 6 months of dosing.

Blood samples were taking at 5, 10, 30, 60 min and 2 and 4 hours after dosing. A maximum of four blood samples was taken from each animal before sacrifice.

Results:

Mean pharmacokinetic parameters are given below.

Dose (µmol/kg)	Day of sampling	C_{max} (µmol/L)		AUC_{0-tau} (µmol·h/L)		CL/F (L/h·kg)		$t_{1/2}$ (h)	
		M	F	M	F	M	F	M	F
20	1	0.80	1.42	0.469	0.576	42.7	34.6	0.50	0.56
	14	0.67	1.74	0.926	0.850	21.7	23.5	0.89	0.52
	90	0.78	0.76	0.532	1.162	37.7	17.3	0.54	1.12
	181	0.65	3.07	0.510	1.654	39.2	12.2	0.74	0.99
40	1	0.93	1.94	0.872	1.108	45.9	36.8	0.82	0.65
	14	1.52	2.56	1.543	1.736	26.2	23.0	1.20	0.66
	90	1.65	1.85	1.718	1.439	23.3	28.0	0.79	0.87
	181	1.17	3.61	2.139	2.761	18.7	14.4	1.15	0.69
80	1	4.82	4.94	2.958	3.041	27.0	26.5	0.91	0.61
	14	1.71	5.15	1.729	3.764	46.5	21.5	1.13	0.86
	90	3.93	2.61	4.751	3.520	16.9	22.8	1.24	0.89
	181	1.55	3.91	2.026	5.389	39.7	14.9	0.69	0.85

The mean peak plasma concentration (Cmax) and area under the plasma concentration Vs time curve (AUCinf) increased with dose. There were higher plasma concentration levels in females rats than male rats.

Test compound: S-PPX (LEA105), metabolite of ropivacaine.
Formula: ———————————
Batch no.: 103/96, source – Astra Pain Control AB, Sodertalje, Sweden.
Animal: Sprague-Dawley male and female rats, approx. 2 months old,
Males 288-305 g and females 250-273 g body weight.
Supplier: ———————————
Dosage: 500 µmol/kg, 4 ml/kg, single subcutaneous administration.
GLP/QA statements: Yes
Experimental procedures:

Animal no	Body weight (g)	Dose vol. (ml)	Dose (µmol/kg)	Dose (µmol)
M101	305	1.2	484	148
M102	296	1.2	499	148
M103	303	1.2	487	148
M104	288	1.2	513	148
M105	292	1.2	505	147
F201	252	1.0	488	123
F202	255	1.0	482	123
F203	252	1.0	488	123
F204	273	1.1	496	135
F205	250	1.0	492	123

Blood sampling: 15, 30, 60, 120, and 180 minutes after dose.

Results: Total and free plasma concentration (µM), protein binding (%) and fraction unbound (fu%) are presented below.

Time (hours)	Mean Total conc. µM	Mean Free conc. µM	Mean Protein binding, %	Mean fu %
0.25	17.4	13.0	25.8	74
0.5	21.1	15.6	26.3	74
1	35.0	26.8	23.4	77
2	23.6	18.2	23.1	77
3	17.1	12.7	24.9	75

Test compound: [^{14}C] LEA103, specific activity – [redacted]
radiochemical purity [redacted] chemical purity [redacted]
source – Astra Arcus AB, Sweden.
Testing facility [redacted]

Animals: Male (181-210 g body weight) and pregnant female (day 18 of gestation) Lister Hooded pigmented rats, supplier – [redacted]
Dose administration: 10 μmol/kg (3.1 mg/kg) administered intravenously.
Date: April 1997
GLP/QA statements: Yes
Sponsor: Safety Assessment, Astra AB.

Experimental procedure: Male animals were killed at 5, 15 minutes and at 1, 4, 16, 48, 192 and 720 hours post dose. Pregnant females were sacrificed at 5and 15 minutes and at 1, 4, 16 and 48 hours post dose. Several sections from each rat were chosen for phosphor imaging. Triplicate measurements were made for each tissue.

Results:

Gender	Time of sacrifice	Tissue	Radio activity (ng equivalent/g)
Male	5 min	Eye (pigmented layer)	58000
		Kidney cortex	33000
		Preputial gland	19000
		Frontal cortex	9700
		Dorsal hippocampus	7000
		Posterior cortex	5900
		Liver	6700
		Submaxillary salivary gland	14000
		Nasal mucosa	15000
		Stomach wall	16000
Female	Similar to male		
		Fetal eye lens	3200
		Fetal eye (pigment)	2600
		Fetal brain	1100
		Fetal liver	1400

Test Compounds: Ropivacaine (LEA103), batch no. 200/93, Purity - [redacted], supplier – Astra
$[^2H_3]$-ropivacaine (LEA136), batch no. QA686/04, supplier – Astra Pain Control
Animal: Beagle dogs (2 M & 2 F), 11.5 – 12.6 kg body weights, supplier – [redacted]

Dosage and administration: 10 µmol/kg (2 ml/kg) $[^2H_3]$-ropivacaine (LEA136) intravenously on days 1 and 29, and
30 µmol/kg (9.9 mg/kg) ropivacaine (LEA103) subcutaneously once daily between days 1 and 30[I month].
Blood samples: On days 1, 8, 15, 22 and 29 of dosing.
GLP/QA statements: Yes
Study dates: October 7 – Nov. 28, 1994.

Results:

Compound / Route	Day	T_{max} (h)	C_{max} (µmol/l)	$T_{1/2}$ (h)	AUC_{last} (µmol.h/l)	AUC_{INF} (µmol.h/l)	CL (l/h.kg)
$[^2H_3]$-ropivacaine / IV	1	0.23± 0.0	5.52± 1.5	0.74± 0.2	3.91± 0.6	4.05± 0.6	2.60± 0.4
$[^2H_3]$-ropivacaine / IV	30	0.23± 0.1	6.49± 2.4	0.75± 0.18	4.46± 0.5	4.60± 0.4	2.24± 0.3
[2H3]-PPX / IV	1	0.90± 0.6	0.051± 0.03	3.3± 4.5	0.103± 0.05	0.183± 0.04	59.5± 17.0
[2H3]-PPX / IV	30	1.29± 0.6	0.064± 0.03	1.6± 1.6	0.145± 0.08	0.182± 0.06	61.7± 25.5
Ropivacaine / SC	1	0.55± 0.14	5.25± 2.36	20.86± 28.65	13.43± 2.15	50.6± 59.1	1.26± 0.94
Ropivacaine / SC	30	0.42± 0.14	6.03± 2.93	1.91± 0.77	17.15± 2.89	19.43± 3.77	1.54± 0.18
PPX / SC	1	2.23± 0.82	0.11± 0.06	8.8± 9.3	0.428± 0.202	0.912± 0.787	49.7± 27.8
PPX / SC	30	2.98± 2.18	0.137± 0.077	2.8± 1.3	0.611± 0.326	0.732± 0.328	52.8± 38.8

There were no differences in pharmacokinetic parameters obtained on day 1 compared to after one month.

in

Animals: Beagle dogs, 13.4 – 16.2 kg body weight, 13 – 16 months old, supplier

Test compounds: Ropivacaine hydrochloride monohydrate (LEA103), ropivacaine hydrochloride (LEA101) and ropivacaine base (LEA132).

Dosage: Each animal received 7 administration with a washout period of one week between treatments.

ID No (Tattoo)	Treatment Formulation (batch)	Date of experiment	Body wt. kg	Volume g	Dose µmol
1026AK	Form 1, (105-4/1-1)	931013	16.2	10.43	175.4
"	Form 2, (105-5/1-1)	931020	16.2	10.19	172.4
"	Form 3, (105-6/1-1)	931027	15.9	10.58	178.3
"	Form 4, (105-7/1-2)	931103	15.9	10.42	164.9
"	Form 5, (1433-1/1-1)	931117	16.3	10.02	162.5
"	Form 6, (PC31027)	-	-	-	-
"	Form 7, (PC31123)	931125	15.8	9.05	145.1
1060AK	Form 1, (105-4/1-1)	931013	13.4	10.92	183.7
"	Form 2, (105-5/1-1)	931020	13.3	10.03	169.7
"	Form 3, (105-6/1-1)	931027	13.4	10.27	173.0
"	Form 4, (105-7/1-2)	931103	13.5	10.43	165.1
"	Form 5, (1433-1/1-1)	931117	13.6	9.83	159.4
"	Form 6, (PC31027)	931110	13.2	9.89	151.4
"	Form 7, (PC31123)	931125	13.6	9.41	150.8
2C188	Form 1, (105-4/1-1)	931013	13.5	10.61	178.5
"	Form 2, (105-5/1-1)	931020	13.9	10.63	179.9
"	Form 3, (105-6/1-1)	931027	13.8	10.34	174.2
"	Form 4, (105-7/1-2)	931103	14.0	10.51	166.4
"	Form 5, (1433-1/1-1)	931117	14.1	9.86	159.9
"	Form 6, (PC31027)	-	-	-	-
"	Form 7, (PC31123)	931125	14.2	9.05	145.1
2C191	Form 1, (105-4/1-1)	931013	13.4	10.61	178.5
"	Form 2, (105-5/1-1)	931020	13.6	10.21	172.8
"	Form 3, (105-6/1-1)	931027	13.7	10.28	173.2
"	Form 4, (105-7/1-2)	931103	14.5	10.37	164.2
"	Form 5, (1433-1/1-1)	931117	14.5	10.06	163.2
"	Form 6, (PC31027)	931110	14.5	10.28	157.4
"	Form 7, (PC31123)	931125	14.5	9.63	154.4

- not treated

Formulations:

Compound	*Form. 1	Form. 2	Form. 3	Form. 4	Form. 5	Form. 6	Form. 7
LEA103 (mg/g)	5.53	5.56	5.54	5.20			
HPMC 4,000 cps (mg/g)	24.5	24.5	24.5	10.0			

Compound	*Form. 1	Form. 2	Form. 3	Form. 4	Form. 5	Form. 6	Form. 7
LEA132 (mg/g)	-	-	-	-	4.45	4.2	4.4
Carbomer 934P (mg/g)					5.0		
Propylene glycol (mg/g)					2.0		
Tween 20	-	-	-	-	?		
Soya-PC347 (mg/g)	-	-	-	-		10	
Sphingolipid (Ba T30615 (mg/g)							149
Palm oil (mg/g)							847
Batch #	105-4/ 1-1	105-5/ 1-1	105-6/ 1-1	105-7/ 1-2	1433-1/ 1-1	PC310 27	
pH	5.7	4.2	6.75	5.9	7.2	?	?

*Form - Formulation

Blood samples: Blood samples were withdrawn from jugular vein before dosing and 0.25, 0.50, 1.0, 2.0, 4.0, 6.0, 8.0, and 24 hours after administration.

GLP/QA statements: Yes

Results:

Formulation	Dose (µmol)	C_{max} (µmol/l)	t_{max} (h)	AUC $_{0\text{-}Tn}$ (µmol.h/l)	AUC $_{total}$ (µmol.h/l)	$T_{1/2}$ (h)	$F_{(0\text{-}Tn)}$ (%)
1	179	0.46± 0.11	1.13± 1.01	0.96± 0.87	1.03± 0.90	0.71± 0.33	8.60± 8.73
2	174	0.39± 0.21	0.88± 0.84	0.82± 0.84	0.89± 0.91	0.96± 0.46	7.71± 8.70
3	175	0.64± 0.37	0.57± 0.32	1.16± 0.73	1.22± 0.73	1.10± 0.16	10.29± 7.18
4	165	0.56± 0.21	0.50± 0.35	0.91± 0.65	1.05± 0.60	0.95± 0.44	8.82± 7.01
5	161	0.36± 0.09	0.43± 0.20	0.67± 0.50	0.78± 0.55	1.42± 0.89	6.74± 5.52
6*	151, 157	0.10, 0.35	1.02, 1.00	0.59, 0.95	1.43, 1.48	9.64,# 2.49#	5.49, 9.34
7	149	0.35± 0.22	1.31± 0.85	1.16± 0.59	1.51± 0.48	3.49± 2.76@	12.20± 6.47

* N=2, individual values # - statistically significant than in formulations 1, 2, and 4
@ - statistically significant than in formulations 1, 2, 3, 4, and 5.

Large individual variations were seen in PK values between animals in all groups studied.

Test compounds: LEA103 – Ropivacaine
LEA131 - Bupivacaine
Animals: Beagle dogs, 8.8-12.2 kg (males) and 7.6-9.9 kg (females), 6-9 months old.
Dosage:

Group	Animal	Compound	Inf. rate ml/kg·h	Dose mg/kg·h	Dose µmol/kg·h
1	51-53 M	saline	0.083	0	-
	54-56 F	saline	0.083	0	-
2	57-59 M	ropivacaine	0.083	0.17	0.53
	60-62 F	ropivacaine	0.083	0.17	0.53
3	63-65 M	ropivacaine	0.083	0.35	1.1
	81 M	ropivacaine	0.083	0.35	1.1
	66-68 F	ropivacaine	0.083	0.35	1.1
	82 F	ropivacaine	0.083	0.35	1.1
4	69-71 M	bupivacaine	0.083	0.17	0.51
	72-74 F	bupivacaine	0.083	0.17	0.51
5	75-77 M	bupivacaine	0.083	0.35	1.0
	78-80 F	bupivacaine	0.083	0.35	1.0

Duration: Continuous epidural infusion for 4 weeks
Blood sampiing: Blood samples (5 ml) were collected before dosing and 1, 3, 6, 12, 24, 48, and 72 hours after the start of dosing. Blood samples were also collected on day 10, 17 and 24 and at the end of the infusion.
Statistical analysis: Stat View® and ANOVA factorial tests.
Study dates: June-July 1994.
GLP/QA statements: Yes

Results:

One male and one female in high dose ropivacaine group were sacrificed in morbid condition

Group (μmol./kg.hr)	T_{max} (h)	C_{max} (μmol/l)	C_{ss} (μmol/l)	Cl (ml/min.kg)	$t_{1/2}$ (h)	AUC 0-24-day (μmol.h/l)	AUC 0-total (μmol.h/l)
0.53 – ropivacaine	104.0± 89.4	0.52±* o.11	0.39*± 0.06	23.9± 0.38	0.92± 0.40	225.1±* 47.8	282.4±* 47.4
1.1 – ropivacaine	145.0± 214.8	0.91±* 0.20	0.76±* 0.16	25.3± 5.9	2.51±* 1.64	427.8*± 74.0	514.8±* 107.4
0.51 – bupivacaine	188.0± 165.9	0.58±* 0.10	0.42±* 0.05	22.4± 2.5	0.59± 0.12	244.9±* 24.0	290.2±* 24.6
1.0 – bupivacaine	84.0± 67.5	1.20±* 0.40	0.88±* 0.24	22.0± 5.6	0.76±* 0.21	484.4±* 97.5	530.5±* 122.9

All statistical analyses described above were performed on the combined male and female data.

The only statistically significant differences in PK values between ropivacaine and bupivacaine treatments were in $t_{1/2}$.

[redacted]

Test compounds: LEA103 (ropivacaine), batch no. 200/93, source – Astra.
LEF244 (fentanyl citrate), batch no. 21777, source – Astra Pain Control.
Animals: Beagle dogs [2 M & 2 F per group], supplier - [redacted]

Group	Compound	Dose (μmol/dog)
1	Saline	-
2	Ropivacaine	58
3	Fentanyl	0.13
4	Ropivacaine + Fentanyl	19 + 0.044
5	Ropivacaine + Fentanyl	58 + 0.13

Duration: Single epidural injection (3 ml/dog)
Blood sampling: Predose and 0.08. 0.17, 0.33, 0.67, 1, 2, 3, and5 hours after dosing.
GLP/QA statements: Yes

. Results:

Group/ Drug	T_{max} (h)	C_{max} (kg/l)	$T_{1/2}$ (h)	AUC_{last} (nmol/l.h)	AUC_{inf} (nmol/l.h)	Cl (ml/min.kg)	V_{dis} (l/kg)
3/Fent.	0.10± 0.05	0.083± 0.025	6.8± 3.1	1.8± 0.49	4.2± 1.2	42.2± 10.6	23.0± 6.8
4/Fent.	0.10± 0.05	0.059± 0.008	0.40	0.07	0.13	510	16.6
5/Fent.	0.08	0.063± 0.011	5.9± 0.85	1.1± 0.15	2.4± 0.11	91.8± 39.0	37.0± 6.2
2./Ropi	0.15± 0.05	0.34± 0.12	3.2± 0.47	2.9± 0.71	4.2± 0.79	19.4± 4.5	5.4± 1.9
4/Ropi	0.17± 0.12	0.52± 0.05	1.7± 0.35	1.6± 0.76	2.1± 0.88	13.5± 4.5	2.0± 0.47
5/ROPI	0.1± 0.05	0.40± 0.10	2.4± 0.74	4.0± 1.4	5.2± 1.7	16.4± 6.7	3.3± 0.94

Test Compounds: LEA103 (ropivacaine), batch no.200/93 and LEF244 (fentanyl citrate), batch no. 21772, supplier – Astra, Sweden.

Animals: Four Male and 4 female beagles _____ Sweden.

Blood Sampling: From jugular vein at predose, 0.25, 0.5, 1, 2, 4, 6, and 24 hours after

Group 1				
Animal ref. no.	Sex	Ropivacaine daily dose [µmol/kg]	Fentanyl daily dose [µmol/kg]	Experimental period
5614	male	10	0.045	Days 0-2
5614	male	20	0.090	Days 6-8
5615	male	10	0.045	Days 0-2
5615	male	20	0.090	Days 6-8
5616	female	10	0.045	Days 0-2
5616	female	20	0.090	Days 6-8
5617	female	10	0.045	Days 0-2
5617	female	20	0.090	Days 6-8
Group 2				
Animal ref. no.	Sex	Ropivacaine daily dose [µmol/kg]	Fentanyl daily dose [µmol/kg]	Experimental period
5618	male	15	0.0675	Days 0-4
5619	male	15	0.0675	Days 0-4
5620	female	15	0.0675	Days 0-4
5621	female	15	0.0675	Days 0-4

dosing on the first and last day of treatment.
GLP/QA statements: Yes

Results:

	Ropivacaine				
	Dose	C_{max}	t_{max}	$T_{1/2}$	Cl.
	(μmol/kg)	(μmol/l)	(h)	(h)	(ml/min*kg)
Group 1	10	2.4±0.53	0.5	-	-
	20	5.8±1.1	0.5	-	-
Group 2	15 (on Day 0)	3.5±1.1	0.5	10.2±10.0	16.0±3.6
	15 (on Day 4)	3.6±1.4	0.38±0.14	4.3±1.2	16.5±1.8

	Fentanyl				
	Dose	C_{max}	t_{max}	$T_{1/2}$	Cl.
	(μmol/kg)	(nmol/l)	(h)	(h)	(ml/min*kg)
Group 1	0.045	1.6±0.64	0.5	-	-
	0.09	5.2±1.2	0.5	-	-
Group 2	0.0675 (on Day 0)	2.9±1.5	0.5	8.3±7.1	71±27
	0.0675 (on Day 4)	2.8±1.3	0.44±0.13	-	69±34

*not applicable

Test compounds: LEA103 (ropivacaine), batch no.201/94 and LEF244 (fentanyl citrate), batch no. 21772, supplier – Astra, Sweden.
Animals: Two male and 2 female beagle dogs, 8.5 month old, 9-12 kg body weight.
Dosing: In cephalic vein once a day for up to 3 hours at 4 occasions, washout period between each dose – 5-7 days.
Blood Sampling: At the end of infusion, and 1 and 3 hours after dosing.
Analytical Analysis:
Study Dates: 25 October – 15 November 1995.
GLP/QA statements: Yes

Animal ref. no.	Sex	Exp. date	Compound	Dosing rate (μmol/h•kg)	Volume (mL/h•kg)	Dose (μmol/kg)
5179	male	951025	Ropi+Fent	1.3 + 0.0024	0.2	3.8 + 0.0072
5180	male	951025	Ropi+Fent	1.3 + 0.0024	0.2	3.8 + 0.0072
5181	female	951025	Ropi+Fent	1.3 + 0.0024	0.2	3.8 + 0.0072
5182	female	951025	Ropi+Fent	1.3 + 0.0024	0.2	3.8 + 0.0072
5179	male	951102	Ropi+Fent	3.8 + 0.0072	0.6	12 + 0.022
5180	male	951102	Ropi+Fent	3.8 + 0.0072	0.6	12 + 0.022
5181	female	951102	Ropi+Fent	3.8 + 0.0072	0.6	12 + 0.022
5182	female	951102	Ropi+Fent	3.8 + 0.0072	0.6	12 + 0.022
5179	male	951108	Ropi+Fent	12 + 0.022	1.8	35 + 0.065
5180	male	951108	Ropi+Fent	12 + 0.022	1.8	35 + 0.065
5181	female	951108	Ropi+Fent	12 + 0.022	1.8	35 + 0.065
5182	female	951108	Ropi+Fent	12 + 0.022	1.8	35 + 0.065
5179	male	951115	Ropi+Fent	35 + 0.065	5.4	20 + 0.038*

* infusion time 0.58 h, in all other cases 3.0 h.

Results:

	Ropivacaine		Fentanyl	
Dosing rate (μmol/h•kg)	C_{max} (μmol/kg)	C_{max}/d_a (kg•h/L)	C_{max} (nmol/kg)	C_{max}/d_a (kg•h/L)
1.3+0.0024	0.84±0.21	0.22±0.06	0.47±0.14	0.066±0.019
3.8+0.0072	3.60±1.42	0.30±0.12**	1.53±0.31	0.070±0.014*
12+0.022	7.25±3.80	0.21±0.11**	3.54±0.39	0.055±0.006*
35+0.065	8.86	-	5.82	-

*P<0.05; **P<0.01

No differences in C_{max} and plasma concentration vs. time data were observed between male and female animals.

Test Compounds: LEA103 (ropivacaine, batch no. 201/94, supplier – Astra.
LEA131 (bupivacaine), batch no. 83404-01, supplier Astra.

LEF244 (fentanyl citrate), batch no. 21772, supplier – Astra.
Animals: Six male and six female beagle dogs.
Dosing: A step up dosing procedure was used as shown in the table.
Washout period: Four days between each infusion period.
Duration: Continuous infusion for 50 or 72 hours.
Route: epidural

Group	Occasion	Dose levels (µmol/h•kg)
1	1	0.53(R) + 0.0010(F)
	2	0.80(R) + 0.0015(F)
	3	1.2(R) + 0.0022(F)
	4	1.2(R) + 0.00056(F)
2	1	0.32(B) + 0.0013(F)
	2	0.71(B) + 0.0028(F)
	3	1.1(B) + 0.0042(F)

Blood Sampling: Predose and 3, 6, and 24 h during infusion, prior to stopping the infusion and 2 hours after stopping the infusion.
Study Dates: November 21 to December 22, 1995
GLP/QA Statements: Yes

Results:

Group	Dose levels (µmol/h•kg)	Css (µmol/L)	Cl (mL/min•kg)
1	0.53(R)	0.26±0.01	33.9±1.9*
	0.80(R)	0.42±0.05	32.3±3.7
	1.2(R)	0.66±0.04	30.5±1.9*
	1.2(R)	0.66±0.08	30.5±3.9
2	0.32(B)	0.26±0.04	21.3±3.2
	0.71(B)	0.65±0.25	20.7±8.6
	1.1(B)	0.89±0.20	21.6±5.5

*P<0.05
These values are in agreement with those found in other intravenous and epidural studies in dogs.

b. Biotransformation

Test Compound: LEA103 (ropivacaine), batch no. 472-31/1-1.
Reference Compounds: LEA144

LEA145

LEA166

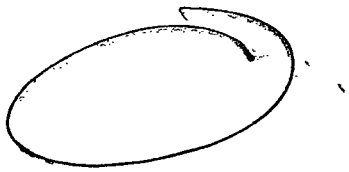
RAD111

were supplied by Astra Draco, Lund, Sweden.

Animals: Male and female Sprague Dawley albino rats, 8-10 weeks old, ~200 g body weight.
Supplier:
Dosing: Four days, subcutaneously.

Group #	Number of animals	Compound	Dose
1	3 M & 3F	LEA103	60 µmol/kg
2	2 M & 2 F	Saline	

The animals were sacrificed on day 5, livers quickly removed and stored.
Enzymatic Determinations: Cytochrome P-450, cytochrome b5, NADPH cytochrome-p450 reductase activities were determined in liver microsomes.



Substance	Name	R_1	R_2	R_3	R_4
LEA103	ropivacaine	C_3H_7	H	H	CH_3
LEA144	4-OH-ropivacaine	C_3H_7	H	OH	CH_3
LEA145	3-OH-ropivacaine	C_3H_7	OH	H	CH_3
RAD111	PPX	H	H	H	CH_3
LEA140	3-OH-PPX	H	OH	H	CH_3
LEA142	4-OH-PPX	H	H	OH	CH_3
LEA166	2-OH-methyl-ropivacaine	C_3H_7	H	H	CH_2OH

The metabolisms of LEA103, testosterone, chlorzoxazone and 7-ethoxyresorufin were also measured.

Results:

Treatment	Cytochrome P450 (nmol/mg)	Cytochrome b5 (nmol/mg)	NADPH-cytochrome P450 reductase [a]
LEA103 male (n=3)	0.69 ± 0.11	0.43 ± 0.06	101.3 ± 15.8
saline male (n=2)	0.87	0.42	104.0
LEA103 female (n=3)	0.64 ± 0.07	0.40 ± 0.01	92.6 ± 5.41
saline female (n=2)	0.64	0.48	97.9

[a] The activity is expressed as nmol/min/mg reduced cytochrome C

There were no differences in micosomal activity between ropivacaine and saline treated rats. Different prototype inducer of P450 enhanced the metabolism of LEA103. The metabolic pattern of testosterone was not changed by LEA 103 treatment.

Test compounds: LEA103 (ropivacaine) and reference substances

Microsomal Sources: Human liver microsomes - supplied by

Study Dates: Feb. – Sept. 1994

Results:

Km and V_{max} values for the NADPH-dependent formation of four identified metabolites of LEA103 in pooled human liver microsomes are giving below.

Metabolite	Km (μM)	Vmax (pmol/mg/min)
LEA144	393	105
LEA145	16	46
LEA166	485	9
RAD111	406	1847

There was a large interindividual variability in the metabolite pattern but all converted LEA103 to the same metabolites.

The metabolic pattern in liver slices prepared from a white male was similar to the profile in the earlier study using human liver microsomes.

Test Compounds: LEA103 (ropivacaine), batch no. 200/93, source - Astra

Study Dates: Sept. to Dec. 1996.

Results:

Effect of ropivacaine on the formation of 6β-Hydroxytestosterone from testosterone (catalyzed by CPY3A4) in human liver microsomes is shown below.

Concentration of LEA103 (mM)	Inhibition (%) 80 μM testosterone	200 μM testosterone
0	0	0
0.01	3	2
0.1	31	18
1	67	56

Ropivacaine is a competitive inhibitor of CYP3A4 with a K_i of approximate 0.52 mM.

Test Compounds: LEA103 (ropivacaine), batch no. 200/93, source – Astra.
LEA131 (bupivacaine), batch no. 54272-02, source – Astra.
Caffeine, methoxyresorufin, 1,7-dimethylxantine, and resorufin supplied by

Results:

Inhibition by bupivacaine on the formation of 1,7-dimethylxanthine from caffeine in human liver microsomes.

Concentration of bupivacaine (mM)	Inhibition of(%) 0.5 mM Caffeine	2mM Caffeine
0.01	63	46
0.125	87	78
1	88	81

Inhibitory effect of ropivacaine on the formation of resorufin from methoxyresorufin in human liver microsomes

Concentration of LEA103 (μM)	Inhibition (%) Methoxyresorufin (0.1μM)	Methoxyresorufin (0.5μM)	Methoxyresorufin (3.0μM)
10	23	9	13
50	56	42	41
100	68	54	54

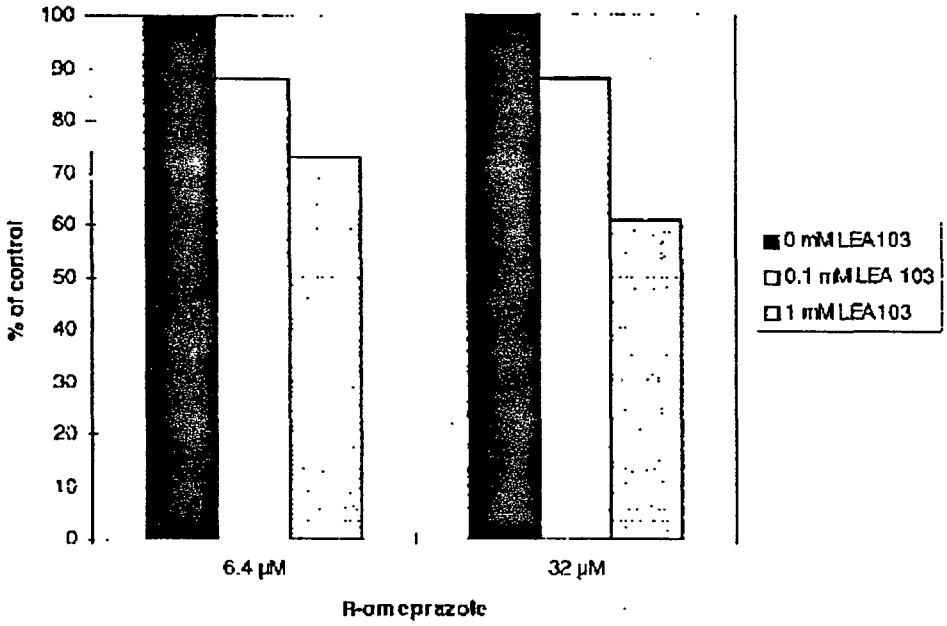
Both compounds showed competitive inhibition of CYP1A2 in human liver microsomes

Test Compounds: LEA103 (ropivacaine, batch no. 200/93, H199/19 (R-omeprazole), H195/80 (hydroxyomeprazole) and H153/52 (internal standard) were supplied by Astra Hassle AB, Molndal, Sweden

Microsomal Source: Pooled human liver microsomes were obtained from

Experimental Procedures: Pooled human liver microsomes were incubated with test compounds after a preincubation with ropivacaine. The incubation mixtures were extracted and analyzed.

Results: The formation of 6-Chlorzoxazone from chlorzoxazone catalyzed by CYP2E1 in human liver was not affected by 1mM ropivacaine. Similarly the formation of 4-OH diclofenac from diclofenac catalyzed by CYP2C9 was not affected by the presence of 1mM ropivacaine in the incubation mixture.



Ropivacaine did inhibit the formation of hydroxy-omeprazole from r-omeprazole catalyzed by CYP2C19 as shown above.

Test Compounds: LEA103 (ropivacaine), LEA145 (3-OH-ropivacaine), RAD111 (PPX), LEA131 (bupivacaine), LEA107 (r-bupivacaine), LEA106 (S-bupivacaine) were supplied by Astra.

respectively.

Experimental Procedures: Pooled human liver microsomes were incubated with test compounds after a preincubation with ropivacaine. The incubation mixtures were extracted and analyzed.

Results: The inhibitory effect of LEA103 on the formation of dextrorphan from 1 to 50 μM dextromethorphan in human liver microsomes is shown below.

Concentration of LEA103 (μM)	Inhibition (%) dextromethorphan				
	1 μM	3 μM	5 μM	25 μM	50 μM
0	0	0	0	0	0
1	10	14	12	4	1
5	24	28	19	4	0
50	88	83	77	51	36

The inhibitory effects of two main metabolites (LEA145 and RAD111) are compared with parent compound (LEA103) on the formation of dextrophan are shown below.

Concentration of LEA103 (μM)	Inhibition (%) dextromethorphan				
	1 μM	3 μM	5 μM	25 μM	50 μM
0	0	0	0	0	0
10	52	43	40	18	18
100	91	91	89	72	55
1000	98	99	98	97	95

Concentration of LEA145 (μM)	Inhibition (%) dextromethorphan 1 μM	3 μM	5 μM	25 μM	50 μM
0	0	0	0	0	0
10	37	35	26	14	10
100	83	79	74	48	40
1000	100	98	97	93	88

Concentration of RAD111 (μM)	Inhibition (%) dextromethorphan 1 μM	3 μM	5 μM	25 μM	50 μM
0	0	0	0	0	0
10	35	33	32	11	6
100	84	80	77	51	33
1000	97	97	96	89	82

Inhibitory effect of LEA131(bupivacaine) on the formation of dextrorphan from dextromethorphan

Concentration of LEA131 (μM)	Inhibition (%) dextromethorphan 1 μM	3 μM	5 μM	25 μM	50 μM
0	0	0	0	0	0
1	54	44	31	0	0
5	83	79	72	45	36
50	97	96	96	89	82

The Ki values for bupivacaine, R-bupivacaine and S-bupivacaine were 1, 2 and 1μM, respectively.

Post mitochondrial supernatant (S9) from male Sprague-Dawley rats was obtained from

Results:

Metabolite	Arochlor induced S9		Control S9		Arochlor/
	UV-area	%	UV-area	%	Control
LEA144	169.0	6	ND	<1	-
LEA145	191.9	7	157.5	18	1.2
RAD111	1158.6	44	410.8	47	2.8
LEA166	754.4	29	171.8	20	4.4
Met. A	122.2	5	18.2	2	6.7
Met. B	117.1	5	63.2	7	1.9
Met. C	59.0	2	40.6	5	1.5
Met. J	52.0	2	17.6	2	3.0
Total	2624.2	100	879.7	101	

ND not determined

The major metabolite formed in both S-9 preparations was RAD111.

Test Compound: Ropivacaine (LEA103), batch no. 472-31/1-1, very stable, Source – Astra.
Species: Dog. Strain: Beagle, 11.5 – 12.5 kg body weights.
Supplier _____
Dose: 30 µmol/kg, 2 ml/kg
Route and Duration: Subcutaneously, once daily for 28 days.
Experimental procedure: Liver microsomes were prepared from treated (4) and untreated (1) dogs. Cytochrome P450 and protein determinations were done by the standard methods.
Study Dates: February 1995 to January 1996.

Results:

Repeated administration of ropivacaine for one month did not change the P450-content of the livers as shown in the table below.

Microsomes	Cytochrome P450 (nmol/mg)
Ropivacaine, male (n=2)	0.68 (0.61; 0.75)
Ropivacaine, female (n=2)	0.67 (0.61; 0.75)
Control, male (n=1)	0.69

There were no differences in the metabolism of ropivacaine in the treated and untreated liver microsomes.

Metabolite	Ropivacaine, male (n=2)	%	Ropivacaine, female (n=2)	%	Control, male (n=1)	%
LEA144	23.5 (21.2, 25.7)	2	30.3 (27.1, 33.5)	3	24.4	3
LEA166	27.7 (26.6, 28.8)	3	29.8 (24.6, 35.0)	3	24.7	3
LEA145	149.5 (141.1, 158.1)	14	148.0 (144.9, 151.4)	14	159.0	20
RAD111	842.5 (841.4, 843.5)	81	822.5 (795.3, 849.8)	80	586.0	74
Total	1043.2	100	1030.6	100	794.1	100

Summary and Evaluation of Pharmacology

LEA103 [S- (-)-1-Propyl-2', 6'-pipecoloxylidide HCl H_20; ropivacaine] is a long acting, local anesthetic agent of the amide type. The pharmacological and toxicological activity of ropivacaine [S- (-)-ropivacaine hydrochloride monohydrate] is similar to that of bupivacaine. Its primary pharmacological applications include epidural anesthesia for the management of postoperative pain, labor pain, and surgical anesthesia. The local anesthetic block of ropivacaine, as with other structurally related local anesthetics, is presumably due to its ability to increase the threshold for electrical excitation in the nerve, by reducing the rate of rise of the action potential, and by slowing the propagation of the nerve impulse.

The FDA approved ropivacaine (Naropin®) on Sept. 24, 1996. The present submission contains supporting preclinical animal studies carried out since approval to support the safety of continuous epidural administration up to one month in analgesic concentrations. The preclinical studies submitted in this supplement have focussed on the evaluation of the metabolism and toxicity of ropivacaine following longer duration of exposure than in the original NDA submission.

In studies comparing ropivacaine and bupivacaine in sensory and motor sciatic nerve block in guinea pigs, the mean onset of sensory blocks were similar between ropivacaine and bupivacaine over a 2.5-10 mg/ml dose range. The R- (+) isomer had a similar onset time. The duration of the motor block was also similar, with bupivacaine showing a significantly longer duration at the lower doses. With the addition of 5 μg/ml of epinephrine to these dosages, prolongation of the block duration occurred with both ropivacaine and bupivacaine. R- (+)-ropivacaine also had a shorter duration of block than ropivacaine. 3-Hydroxy-ropivacaine and 4-hydroxy-ropivacaine, the metabolites, were shown to have some local anesthetic activity.

Lumbar spinal anesthesia block in mice following a single injection was significantly prolonged with bupivacaine than with ropivacaine when compared at the same concentrations (2.5–10.0 mg/ml). In dogs, the time to onset was greater with ropivacaine than bupivacaine at 7.5 mg/ml. With repeated intrathecal doses administered to rats, rapid absorption occurred, followed by rapid elimination. The systemic availability was estimated to be 72% and 102% following single and multiple intrathecal injections, respectively.

Epidural anesthesia was studied in guinea pigs, dogs, sheep and monkeys. The duration of sensory and motor blocks in guinea pigs with and without 10 μg/ml adrenaline were significantly longer with bupivacaine than with ropivacaine at equal mg/ml doses. In dogs, no significant differences were seen between ropivacaine (10 mg/ml) and bupivacaine (7.5 mg/ml) in the incidence, time to onset, duration of block, or time to recovery. In sheep, no significant differences occurred between ropivacaine and bupivacaine in the onset of sensory or motor block; however, the time to complete recovery from the motor block was significantly longer for bupivacaine at 5 and 7.5 mg/ml. The results with monkeys were similar to those in the dog.

The data from the infiltration anesthesia study in guinea pigs showed the duration of

complete block to be longer with ropivacaine than with bupivacaine at 0.25% - 7.5% concentrations, with and without 5 μg/ml epinephrine. The time to complete recovery was also significantly longer with ropivacaine.

Corneal anesthesia in rabbits indicated no differences with 0.5%-1% concentrations in the duration or time of onset of block with ropivacaine and bupivacaine.

In studies comparing R- (+)-ropivacaine, S- (-)-ropivacaine, and RS- (±)-ropivacaine, the S enantiomer produced a longer duration in local anesthesia than the racemate or the R enantiomer, and when compared with bupivacaine, ropivacaine produced a longer block, although not always significant at the equivalent molar dose. In the spinal anesthesia studies (mice, rats, and dogs), the duration of motor block was significantly greater with bupivacaine than with ropivacaine at the same mg/ml dose.

In vitro conduction block studies were carried out with frog sciatic nerves, rat vagus and phrenic nerves, and rabbit vagus nerves. The action potential blocking activity of ropivacaine and bupivacaine were equal in the frog sciatic nerve from 1 μM to 50 μM. All blocks were reversible within 2 hours. The action potential amplitudes of rat vagus and phrenic nerves were reduced with concentrations of 25-200 μM. Conduction block in rabbit vagus nerves (A fiber) with ropivacaine was somewhat less with ropivacaine, compared to equal concentrations of bupivacaine. No differences were found between ropivacaine and bupivacaine on C fibers. The ED_{50} values were larger for ropivacaine than bupivacaine; lidocaine values were two to four times larger than ropivacaine ED_{50} values.

Single epidural administration of ropivacaine and fentanyl caused dose-dependent increase in anesthesia, duration of motor and sensory signs and absent of superficial pain sensation in dogs. Test compounds did not cause any gross and microscopic pathological changes in spinal canal and spinal cord.

The local anesthetic effect of metabolites of ropivacaine was investigated in guinea pig. S-PPX (LEA105) and (R, S)-2-OH-methyl-ropivacaine (LEA166) showed about one quarter of the local anesthetic potency of ropivacaine. R, S-3-OH-PPX (LEA140) at 5 mg/ml showed no local anesthetic effect. The duration of the anesthetic effect of the metabolite of ropivacaine was shorter than that of the parent compound.

To conclude, the above studies indicated that ropivacaine is similar to bupivacaine in its pharmacological activity as a local anesthetic. Ropivacaine was not comparable to lidocaine in many of the preclinical studies.

Summary and Evaluation of Acute, Subchronic, and Chronic Toxicity Studies.

Acute intravenous and subcutaneous studies in mice and rats produced decreased motor activity, irregular breathing, piloerection, tremors, ataxia, clonic convulsions, and death,

which occurred during or shortly following ropivacaine administration. When comparing ropivacaine, bupivacaine, or lidocaine in I.V. or S.C. lethality studies, mortality was of the order bupivacaine > ropivacaine > lidocaine. All mortality was due to convulsions, but not all convulsions led to mortality. Further, the duration of convulsion was not changed with the addition of 1-2.5 μmol/Kg epinephrine. There appeared to be little difference in mortality between each enantiomer and racemic ropivacaine. The estimated fifty percent mortality range seen in mice was 320 -360 μmol/Kg S.C. and 85-60 μmol/Kg I.V. In rats these values were 180-230 μmol/Kg S.C. and 30-37 μmol/Kg I.V. The data show that rat is more sensitive than the mouse to the toxicity of ropivacaine.

Simultaneous subcutaneous administration of ropivacaine and fentanyl showed a synergistic effect of these two test compounds compared to when they were given alone in rats. Lung congestion and alveolar edema were noted in dead animals. Surviving animals had microfocal lung granulomas with accumulation of alveolar macrophages. There were superficial erosions of the glandular stomach mucosa and focal myonecrosis of the heart. Skin lesions at the injection sites, on the back and neck regions, were seen in all treated animals.

An intravenous infusion of 1.8 ml/kg.hr for 3 hours with a combination of 6.4 μmol/ml ropivacaine and 0.012 μmol/ml fentanyl was considered as a maximum tolerated dose in beagle dogs.

Subcutaneous repeat dose studies were conducted in rats for 2 weeks and 1 month. Ropivacaine produced small ulceration and incrustations at the injection sites with all S.C. dose (10-90 μmol/Kg sq. x 2 weeks). Mortality occurred at 90 μmol/Kg (30 mg/Kg). The one-month S.C. study in rats (10-80 μmol/Kg, 3.3-26 mg/Kg) produced short clonic convulsions and cyanosis at 80 μmol/Kg. Other changes were increases in cholesterol and calcium and decreases in potassium and magnesium. No gross pathology was reported, and no histopathologic lesions were seen in the heart.

Subcutaneous repeat dose studies in dogs were conducted for 3 days and 1 month. A 6-month S.C. concomitant rectal administration was also evaluated. The 3 day study (5-50 μmol/Kg, 1.6-16.4 mg/Kg) reported neurologic signs - muscular tonus, rigid movements, mild body tremors, ataxia, and urinary incontinence, and periodic clonic convulsions. No arrhythmias were reported; however, irregular or occasional changes in PR and QRS deviations were seen but were reported as within the normal range. Periodic clonic convulsions occurred in one dog dosed at 50 μmol/kg. These same dogs were doses with bupivacaine (5-50 μmol/Kg, 1.6-16.2 mg/Kg) following a 5 day treatment free washout. Severe body tremors occurred at the high dose. Irregular/occasional changes in PR and QRS that occurred were said to be within the normal range. Severe convulsions occurred in two dogs dosed at 50-μmol/kg bupivacaine. The one month S.C. study (10-40 μmol/Kg, 3.3-13.2 mg/Kg LEA103) produced mostly the same toxic signs. The EKGs picked up deviations in the QRS axis in 1 or 2 dogs at 20 and 40 μmol/Kg, but all were said to show normal sinus rhythms. The ropivacaine no effect dose was 20 μmol/Kg (6.6 mg/Kg). The subcutaneous/rectal administration (10-40 μmol/Kg S.C. plus 320-1300 μmol/Kg LEA103 rectal) resulted in a dose-related increase in feces and itching at the S.C. site. Hb, RBCs,

and PCV were decreased at 20 and 40 μmol/Kg. There were some changes in the lungs (inflammatory changes), urinary bladder (inflammatory changes and focal hemorrhage), pyelitis in all drug groups in the kidneys, liver (inflammatory changes and foci of mononuclear cells), and inflammatory changes in the anal canal. Individual variables were recorded in the EKGs, but not considered as related to the treatment.

Administration of ropivacaine (15 μmol/kg, 4.7 mg/kg) and fentanyl (0.068 μmol/kg, 0.023 mg/kg) subcutaneously for 5 days in a dose finding study in beagle dogs caused depressed behavior and increased salivation for up to 4 hours. No organ weight changes or histological changes were observed at this maximum tolerated dose.

Ropivacaine and bupivacaine were well tolerated by the beagle dogs during a continuous 24 hours epidural infusion at dose levels of 0.17 mg/kg/h (0.53 μmol/kg/h of ropivacaine or 0.51 μmol/kg/h of bupivacaine) and 0.35 mg/kg/h (1.1 μmol/kg/h of ropivacaine or 1.0 μmol/kg/h of bupivacaine) for 4 consecutive weeks. Neurological signs observed were consistent with the pharmacological action of these compounds. Histopathological examination revealed the extension of the inflammation from the epidural and dura mater (ropivacaine groups) into the arachnoid region (bupivacaine group). There were no dose-related effects. There was no evidence of systemic toxicity in any group. The plasma concentrations of ropivacaine and bupivacaine reached a steady state within 6-12 h after the continuous epidural infusion.

Group	Tmax (h)	Cmax (μmol/l)	Css (μmol/l)	CL (ml/min/kg)	T1/2 (h)	AUC 0-24 h (μmol.h/l)	AUC 0-total (μmol.h/l)
Ropi-low dose	104±89.4	0.52±0.11	0.39±0.06	23.9±3.8	0.92±0.4	225±48	282±47
Ropi-high dose	145±215	0.91±0.2	0.76±0.16	25.3±5.9	2.51±1.6	428±74	515±107
Bupi-low dose	188±166	0.58±0.1	0.42±0.05	22.4±2.5	0.59±0.1	245±24	290±25
Bupi-high dose	84±67.5	1.20±0.4	0.88±0.24	22.0±5.6	0.76±0.2	484±98	531±123

Ropi – ropivacaine Bupi - bupivacaine

Continuous epidural infusion of ropivacaine + fentanyl, bupivacaine + fentanyl or fentanyl alone for 4 consecutive weeks caused neurological signs in beagle dogs. There was reasonable dose proportionality for all three-test compounds. The concentrations used were up to 13 μmol/ml (4.0 mg/ml for ropivacaine/bupivacaine and up to 0.024 μmol/ml (8.0 μg/ml) for fentanyl. All groups were infused at a dose volume of 0.083 ml/kg/hour. Treatment of test compounds produced low weight gain and reduced food intake. Heart rate, cardiac rhythm or conduction, hematological, bloods clinical chemistry or urinary parameters and eyes were not effected with treatments. Neurological signs of paresis, paralysis and lameness with losses or changes in reflexes are expected effects of ropivacaine and bupivacaine when given by epidural infusion. Stiffness and urinary retention may be associated with an opioid effect, fentanyl. Comparison between treated groups was difficult due to individual variability. There was no evidence of systemic toxicity or treatment–related changes in the nervous tissue with any of the treatments. Mean plasma concentrations of the ropivacaine (R), bupivacaine (B) and fentanyl (F) at steady state (Css) and clearance (Cl) are given on the next page.

Group	Dose level (μmol/kg.h) R/B	+ F	Days	Css R/B (μmol/L)	F (nmol/L)	Cl R/B (ml/min.kg)	F(ml/min.kg)
2 (low R + F)	0.53	0.001	1 (R+F) 30/31 (R+F)	0.27±0.07 0.35±0.08	0.15±0.013 0.22±0.03	35.2±13.7 26.7±8.1	109.4±8.7 76.1±8.0
3 (high R +F)	1.1	0.002	3 to 30/31(R) 3 (F) 30/31 (F)	0.81±0.23	 0.31±0.06 0.47±0.12	24.7±7.0	 112.9±25.5 74.8±17.6
4 (B +F)	1.1	0.002	3 (B+F) 30/31 (B+F)	1.3±0.23 0.87±0.29	0.35±0.1 0.43±0.06	14.5±2.7 23.0±7.7	100.6±22.1 79.8±13.4
5 (F)	0	0.002	3 (F) 30/31 (F)		0.32±0.07 0.49±0.20		107.3±23.2 94.2±80.4

There were no differences between male and female animals.

The lethal and convulsive doses of metabolites of ropivacaine following intravenous administration were higher than the parent compound in mice indicating that metabolites are less toxic than parent compounds in mice. The behavioral effects of metabolites were similar to ropivacaine. Single dose subcutaneous toxicity of LEA 105 (S-pipecoloxylidide), a primary metabolite of ropivacaine, was investigated in rats. Deaths occurred in males (1/5 at 929 mg/kg) and females (4/4 at 465 mg/kg) within 3 to 5 hours after dosing. There was a dose-related decrease in body weight gain in male but not in female rats. Clinical observation were generally associated with the respiratory (decreased or irregular respiration) and motor (loss of righting reflex and disinclined/unable to move) systems. Adrenal cortical vacuolation was seen in 2/4 treated males at 4000 μmol/kg. LEA105 caused dose-dependent localized irritation at the injection sites.

Systemic toxicity of a single intravenous administration of LEA 105 (S-pipecoloxylidide), a primary metabolite of ropivacaine, was also investigated in rats. Deaths occurred in 2/5 males at 420 μmol/kg, 3/3 females at 540 μmol/kg, 2/2 males and 2/2 females at 650 μmol/kg. The dose range for the maximum non-lethal dose and the minimum lethal dose of ropivacaine in rats was 15-30 μmol/kg. There were no effects on body weight gains during the treatment. Clinical observations were generally associated with the respiratory (decreased or irregular respiration) and motor (loss of righting reflex and unsteady gait) systems. Histopathological examination of surviving animals did not reveal any organ toxicity.

Cardiovascular Toxicity Summary

In conscious rats, the addition of 1.4 μg/Kg of epinephrine to 2.5 mg/Kg ropivacaine I.V. increased the mean arterial pressure, the PQ/QT/ QRS values, and decreased the heart rate above those values reported for 2.5 mg/Kg ropivacaine without added epinephrine. Singular

ectopic beats and 2°AV blocks were present in both groups. Sustained complex arrhythmias, pulmonary edema, convulsions, and deaths were present in the ropivacaine only group. With added epinephrine, convulsions, pulmonary edema, complex arrhythmias, and mortality resulted. The toxicity increased further with increasing amounts of added epinephrine. Deaths were attributed to pulmonary edema and or ventricular arrhythmias.

In study (20) F29, I.V. doses required to cause convulsions in six conscious nonsedated dogs were 14.84 μmol/Kg (4.88 mg/Kg) for ropivacaine, 13.69 μmol/Kg (4.31 mg/Kg) for bupivacaine, and 82.59 μmol/Kg (20.84 mg/Kg) for lidocaine and indicated that bupivacaine was more cardiotoxic than ropivacaine. There were no deaths in any group with convulsive doses. Ventricular arrhythmias were of a greater frequency with bupivacaine than with ropivacaine; no ventricular arrhythmias occurred with lidocaine. Seizure duration was somewhat longer with bupivacaine than with ropivacaine or lidocaine. At 2x the convulsive dose, 1/6 of the ropivacaine and 5/6 of the bupivacaine animals died. There was a greater occurrence of ventricular arrhythmias in the bupivacaine group, with one dog progressing to ventricular fibrillation. No arrhythmias occurred at this dosage in the ropivacaine or lidocaine animals.

[redacted] reports on the treatment of the acute toxicity produced from rapid IV administration of ropivacaine or bupivacaine in conscious dogs. No ventricular arrhythmias were observed with 4.9 mg/Kg ropivacaine or 4.3 mg/Kg bupivacaine, both of which are doses producing convulsions. At 2x the convulsive dose, all 5/6 developing convulsions in the ropivacaine group survived, while two of the 4/6 that developed convulsions in the bupivacaine group could not be saved by intervention. Cardiovascular toxicity and resuscitation was further evaluated in anesthetized dogs [redacted]. Resuscitation was initiated when the systolic BP was <45 mm Hg. The results indicated no great differences in cardiovascular toxicity, mortality, or resuscitation results. Resuscitation required rapid control of the hemodynamic changes and acidosis.

The differences in cardiac safety between bupivacaine, ropivacaine and enantiomers were compared in the perfused hearts from albino rabbits. At least 6 animals per group were tested at 1, 3 and 10 μM of test and reference compounds. All agents showed slow conduction and reduced excitability in a dose-dependent manner.

No significant difference in the convulsive dose was seen in the IV infusion study conducted in pregnant and nonpregnant sheep. Doses producing hypotension, respiratory arrest, or circulatory collapse were similar in both groups. Mean concentration of ropivacaine in the heart and brain were also not significantly different between the two groups, although the mean values were somewhat higher in the pregnant animals. An earlier study (Morishima, HO, et al, Anesthesiology 1985; 63:134-139) reported that doses of bupivacaine required to produce equivalent toxic signs in pregnant sheep were greater than in nonpregnant sheep.

Summary and Evaluation of Reproductive Toxicology Studies

Reproduction studies were performed in rats and rabbits. The daily subcutaneous administration of LEA103 (up to 23 mg/kg) to male Sprague-Dawley rats for 9 weeks before mating and during the mating period, and to female rats for 2 weeks before mating and 42 days post coitus did not effect the mating rate, the conception rate and the length of gestation. An increased number of pups (~25%) were found dead within 3 days post parturition in the high dose group (28→23 mg/kg, 84→71 μmol/kg). There was no significant pup loss after day 3 in any group. Similar results have been obtained with a pharmacologically closely related compound, bupivacaine, at 30 mg/kg (25% loss in pups when compared with controls).

The increase in pup loss in this segment I reproductive study, but not in segment III (peri-and postnatal) study may be due to a longer period of dosing before delivery in segment I (~35 days compared with 6-7 days). As it is also mentioned in the Pharmacokinetic section, subcutaneous administration of 26 mg/kg LEA103 for 10 days showed 25-35% increased plasma concentration compared with single administration in both pregnant and nonpregnant rats ——————————— There were no differences in body weight gain or food consumption between pups in the control and treated groups. The reproduction capacity of the F1-generation was the same in all groups. The F2-generation did not show intergroup differences in body weights, litter size and pup loss in this multigenerational (segment I) study in rats. Therefore, this increase in pup loss may be due to impaired maternal care of the pups (such as nursing performance) caused by an increased plasma concentration of LEA103 in the high dose group.

During the teratology study, daily doses of 16, 32 and 81 μmol/kg (5.3, 11.0 and 26.0 mg/kg) of LEA103 given subcutaneously on days 6-15 of pregnancy in rats did not effect body weight gain, food consumption, preimplantation loss, fetal loss and fetal weights. Plasma half-life was similar in all three groups, about 1.7 hours on day 6 and about 2.4 hours on day 15. The placental transfer of labeled LEA103 was linear with a fetal tissue/maternal blood ratio of 45%, 50% and 46% after 16, 32 and 81 μmol/kg administration, respectively. The corresponding values in the amniotic fluid were 17%, 22% and 21%, respectively. LEA103 at dose levels of 16, 32 and 81 μmol/kg/day during days 6-15 of pregnancy did not effect organogenesis and early fetal development in Sprague-Dawley rats.

LEA 103, similar to other local anesthetic agents such as lidocaine, mepivacaine and bupivacaine, crossed the placenta and reached the fetal circulation in pregnant rabbits during the dose range finding teratology study. Daily doses of 1.3, 4.2, and 13 mg/kg/day (corresponding to 4, 13 and 39 μmol/kg) in rabbits on days 6-18 of pregnancy did not produce any mortality but produced lack of one kidney in two animals in the high dose group. Major skeletal defects (unossified vertebral arches and skull) were found in one pup in the low dose group, two pups in the medium dose group and two pups in the high dose group.

Daily doses of LEA up to 11 mg/kg (32 μmol/kg) from day 15 of pregnancy to day 21 post parturition did not cause adverse effects on the dams or on the litters during peri- and postnatal development in rats. However, two mortalities seen at 26 mg/kg (81 μmol/kg) were probably due to test compound. The high dose did not effect the delivery, lactation or litters.

During a comparative peri- and postnatal study in S-D rats, daily administration of LEA131

(bupivacaine) at 43 or 53 μmol/kg from day 15 of gestation until day 21 post parturition produced piloerection, increased salivation, chewing, irregular breathing and clonic convulsions. The onset of these signs was ~10 minute after dosing and continued up to 5 hours. No toxicological signs were noticed in any animal receiving LEA103 (ropivacaine) up to 75 μmol/kg/day (25 mg/kg). There were no intergroup differences in litter size, litter weight, mean pup weight, and physical development (pinna unfolding, tooth eruption, eye opening). The peak plasma concentration (C_{max}) after the administration of LEA103 or LEA131 was reached within the first sampling interval (30-min). The AUC and C_{max} values on day 20 showed a tendency to increase linearly with dose for both drugs Equimolar doses of LEA103 and LEA131 gave similar PK values. This indicates that LEA103 was less toxic in the pregnant rats than LEA131.

Dose μmol/kg	Compound	C_{max} μmol/L	t_{max} h	AUC $_{total}$ μmol/L.h	$t_{1/2}$ h	F %
16	LEA103	1.09 ± 0.38	0.8 ± 0.3	3.69± 0.48	2.31 ± 0.96	93.02 ± 12.22
43	LEA103	1.79 ± 0.46	1.0 ± 0.9	15.01± 3.77	6.54 ± 2.58	140.93 ± 35.42
75	LEA103	4.83 ± 1.70	0.6 ± 0.2	18.01 ± 2.27	2.67 ± 0.63	96.94 ± 12.24
16	LEA131	0.66 ± 0.16	0.6 ± 0.2	8.74 ± 5.12	14.99 ±12.25	-
43	LEA131	2.17 ± 0.43	0.8 ± 0.3	10.92 ± 0.87	5.16 ± 0.75	-
53	LEA131	3.97 ±1.43	0.5	11.25 ± 1.63	2.73 ± 2.17	-

Summary and Evaluation of Genotoxicology Studies

Mutagenicity studies were conducted in the following tests: 1) Ames Salmonella/mammalian microsome mutagenicity test, 2) mouse lymphoma test, 3) E. coli differential DNA repair test in vitro, 4) analysis of structural chromosome aberration in human lymphocytes, 5) mouse micronucleus test, 6) E. coli host-mediated DNA repair test in mice, and 7) somatic mutation and recombination test in Drosophila melanogaster. Of the above tests, the mouse lymphoma test was positive in the presence of S9 metabolic activation. When the test was repeated using Fisher's medium as the solvent, rather than DMSO as in the first one, the study was positive in the presence of metabolic activator. Hence, it is concluded that ropivacaine is mutagenic in the mouse lymphoma assay. All other tests conducted to evaluate mutagenicity were negative.

Summary and Evaluation of ADME Studies

Ropivacaine's metabolic profile has been studied in mice, rats, rabbits, dogs, and sheep. Urinary metabolites that have been isolated are the 3-hydroxy-ropivacaine and its glucuronide, 4-hydroxy-ropivacaine and its glucuronide, N-despropyl-ropivacaine (PPX), 6-oxo-PPX, and 3-hydroxy-PPX. The protein binding of S-PPX, a metabolite of ropivacaine in humans and animals, was low (19-29%) after single subcutaneous administration in rats and it was independent of concentration in the range of 13-36 μmol/l.

Ropivacaine was rapidly distributed in rats to the tissues following IV administration. The elimination half-life for ropivacaine in the various tissues ranged from 0.57 hours in the heart to 1.27 hours in the kidney. The radioactivity half-life, however, was 2.9 to 19.1 hours; the $t_{1/2}$ in the heart was 15 hours. Radioactivity was also detected in the milk of lactating rats. Ropivacaine was 90% bound to serum protein in the rat. In mice, high levels of radioactivity were in the brown fat, nasal mucosa, gastric mucosa, wall of the eyeball (melanin tissue), adrenals, islets of Langerhans, and kidney. High levels of radioactivity could be detected in the melanin-containing layer in the retina of mice even after 32 days. In dogs, radioactivity was distributed throughout the body following sq. administration. The highest radioactivity was seen in the liver, followed by the kidney and spleen. Tissue half-life for radioactivity was longest in the eyes and pigmented skin (17 days). Pharmacokinetic data for amide-type local anesthetics in dogs after IV infusion (Anesth Analg 1988:67:1053-8) are given below.

	Ropivacaine	Bupivacaine	Mepivacaine	Lidocaine	Etidocaine
$t_{1/2}$ (min)	26	39	45	46	60
V_{dss} (L/kg)	1.1	1.2	1.9	2.3	3.4
Cl (ml/min/kg)	41	32	39	56	58

Ropivacaine was rapidly absorbed after rectal administration in Sprague-Dawley rats. There was no accumulation of the drug after repeated administration for six months. The distribution pattern of [^{14}C] LEA103 following single intravenous administration was similar in Hocded Lister pigmented rats. [^{14}C] LEA103 and/or its metabolite penetrated the blood brain barrier in male and female rats at 5 minutes. In the pregnant female rat [14C] LEA103 and/or its metabolites crossed the placental barrier and fetal exposure was measurable in all tissues until 4 hours after administration. Radioactivity was only evident in the pigmented layer of the eye at 720 hours post-dose.

In pigmented mice the urine and feces distribution was 34% and 53%, respectively. The distribution in rats was 42% in the urine and 52% in the feces. Urine and feces recovery of administered radioactive ropivacaine in dogs by the end of 30 days was 72% and 27%, respectively.

The pharmacokinetics profiles of ropivacaine and PPX, a metabolite of ropivacaine, after subcutaneous and intravenous administration in beagle dogs were not different on day 1 compared to after I month of dosing. Plasma concentration of ropivacaine reached steady state in the first 12 hours during continuous epidural infusion in dogs. Depending on the concentration of ropivacaine infused, the plasma concentration at steady state were between 0.2 – 1.6 μmol/l or 0.05 – 0.4 mg/l. There was no accumulation of the drug during one-month infusion. Ropivacaine in different gel vehicles was rapidly absorbed with a very low bioavailability after rectal administration to dogs. The individual variations were very large. The formulation used in human clinical studies was Formulation 1 (Ropivacaine plus HPMC, pH 5.7).

No significant difference in PK parameters was seen when ropivacaine or bupivacaine was infused epidurally in dogs for four weeks except $t_{1/2}$ values. The results of this study indicate that plasma concentrations of both drugs reached steady state within 6-12 hours after starting the continuous infusion. The pharmacokinetics of ropivacaine after single epidural administration in beagle dogs was probably not effected by the presence of fentanyl. The PK values for ropivacaine and fentanyl did not change over 5-day subcutaneous administration in beagle dogs. Dose level, gender and the presence of fentanyl did not show any effect on the clearance of bupivacaine and ropivacaine at lower dose in beagle dogs. The clearance of ropivacaine at higher dose level (1.2 μmol/h.kg) was lower at the terminal phase. Fentanyl also demonstrated a significantly lower CL at the end of the infusion. The reasons for this lower CL are not known.

Ropivacaine treatment for one month did not change total cytochrome P450 content of the dog liver. This lack of effect on the level of P450 in the livers confirms the absence of differences in the pharmacokinetic profile of ropivacaine after single and one month administration of ropivacaine.

In pregnant and nonpregnant sheep, the PK data resulting from IV administration of ropivacaine followed the trends seen with bupivacaine in pregnant and nonpregnant sheep; however, with all parameters that were compared, the values for ropivacaine were lower. Serum concentrations of ropivacaine were higher in pregnant sheep and protein binding was slightly higher. After epidural administration of ropivacaine and bupivacaine to female sheep, the AUC and $t_{1/2}$ values were lower with ropivacaine.

In addition to the in vivo metabolite studies, in vitro studies were also evaluated with liver microsomes from animals and humans. Metabolic activity is rapid in the liver. Only small amounts (1-3%) of ropivacaine are excreted unmetabolized following IV or S.C. administration to male rats. There was no evidence for the interconversion of the S-(-) to the R- (+) isomer of ropivacaine in the rat, dog, sheep or human.

The treatment of male and female rats with LEA103 for four consecutive days did not change the levels of different cytochrome P450 isozymes. The metabolism of LEA103 was increased by inducers (phenobarbital, β-naphthoflavone) of CYP3A.

Ropivacaine is metabolized to four major metabolites in human liver microsomes. The

NADPH-dependent metabolism to 3-OH-ropivacaine (LEA145) is catalyzed by CYP1A (high affinity). The formation of 4-OH-ropivacaine (LEA144), 2-OH-methylropivacaine (LEA166) and PPX (RAD111) was catalyzed by CYP3A (low affinity). CYP3A4 is the predominant form of P450 in the human liver, at least 30% of the total P450. Many drugs have been shown to be the substrates for CYP3A, e.g. testosterone, progesterone, prednisolone, erythromycin, cyclosporine, lidocaine and diazepam. Therefore, the possibility of clinical drug interactions at an enzyme level exists.

Ropivacaine was a competitive inhibitor of CYP3A4 with a Ki value of approximately 0.52 mM during an in vitro metabolism of testosterone in human liver microsomes.

Ropivacaine and bupivacaine are metabolized by CYP1A2 in human liver microsomes

The formation of 6-OH chlorzoxazone from chlorzoxazone a substrate for metabolism by CYP2E1, and the formation of 4-OH diclofenac from diclofenac a substrate for metabolism by CYP2C9 was not inhibited by the presence of 1 mM ropivacaine in human liver microsomes. Therefore, it is considered that clinical use of ropivacaine will not inhibit these enzymes. However, ropivacaine did inhibit CYP2C19 up to 12% at a concentration of 0.1 mM and up to 40% at 1 mM in human liver microsomes.

Ropivacaine was a competitive inhibitor of CYP2D6 during the conversion of dextromethorphan to dextrorphan in human liver microsomes. The K_i value was approximately 5μM. The plasma concentrations obtained after ropivacaine use is estimated to be 2-8 μM. Therefore, this inhibition is considered to have clinical consequences on drugs being dependent on CYP2D6. The corresponding K_i value for bupivacaine is approximately 1 μM, indicating that bupivacaine may have more clinical effects than ropivacaine

There were no differences in metabolic pattern of ropivacaine in liver post mitochondrial supernatant from untreated and Arochlor-treated rats.

Overall Summary and Evaluation

LEA-103 (ropivacaine HCl monohydrate, Naropin™) is a new, long acting, amide-type, local anesthetic agent with pharmacological properties similar to those of bupivacaine (Marcaine) and mepivacaine (Carbocaine). Unlike these agents, which are racemic mixture, ropivacaine is exclusively the S- (-) enantiomer. The S- (-) form is less toxic and/or has a longer duration of action than the R- (+) form.

Ropivacaine has been shown to be a local anesthetic agent similar in activity to bupivacaine in conduction, infiltration, and topical anesthesia. It is active in peripheral neural block and as a central neural blocker. At equivalent or near equivalent doses, ropivacaine produced longer blockade. Toxicity in general is less with ropivacaine than with equivalent doses of bupivacaine. Of concern is cardiotoxicity, as this drug is of the same class as bupivacaine. The submitted studies reported less cardiotoxicity with LEA103 compared to bupivacaine. However, with high IV doses this drug will produce cardiovascular toxicity similar to that

reported for bupivacaine. Of additional interest is the toxic effect of the drug during pregnancy. Those studies comparing ropivacaine toxicity in pregnant and nonpregnant sheep indicated no increase in toxicity in pregnant ewes.

Ropivacaine at dose levels up to 26 mg/kg/day (156 mg/m^2/day) subcutaneously did not effect fertility, general reproductive performance, teratology, perinatal and postnatal development in S-D rats. Teratogenicity studies in rabbits did not effect organogenesis or early fetal development at dose level up to 13 mg/kg (156 mg/m^2). Ropivacaine was positive in the mouse lymphoma test in the presence of metabolic activation, but other mutagenicity assays were negative.

Ropivacaine rapidly distributes to most organs, particularly to the liver, heart, brain, and lungs. High concentrations of radioactivity remain in the pigmented tissues after administration of labeled drug. Radioactivity passes the blood brain and placental barriers and is found in the milk of lactating rats and in fetal tissue. There is little unmetabolized ropivacaine excreted. It is extensively metabolized and excreted in the urine and feces, the proportion varies with the species. Ropivacaine's principal metabolite is 3-hydroxy-ropivacaine, which is excreted mainly as the glucuronide. Other metabolites include 4-hydroxy-ropivacaine, the N-despropyl-ropivacaine, 2-hydroxy-methyl-ropivacaine, and several other minor metabolites. 3-Hydroxy-and 4-hydroxy-ropivacaine have shown some local anesthetic activity. Ropivacaine is not interconverted to the R-(+) enantiomer.

Epidural studies in dogs submitted in this supplement supported the reasonable safety for the proposed epidural use in humans.

Package Insert Section

Currently approved label:

Pregnancy Category B

"Teratogenicity studies in rats and rabbits did not show evidence of any adverse effects on organogenesis or early fetal development in rats or rabbits. The doses used were approximately equal to 5 and 2.5 times, respectively, the maximum recommended human dose (250 mg) based on body weight. There were no treatment related effects on late fetal development, parturition. Lactation, neonatal viability or growth of the offspring in 2 perinatal and postnatal studies in rats, at dose levels up to approximately 5 times the maximum recommended human dose based on body weight. In another study with a higher dose, 23 mg/kg, an increased pup loss was seen during the first 3 days postpartum, which was considered secondary to impaired maternal care due to maternal toxicity.
There are no adequate and well-controlled studies in pregnant women of the effects of Naropin on the developing fetus. Naropin should be used during pregnancy only if clearly needed. This does not preclude the use of Naropin after fetal organogenesis is completed or for obstetrical anesthesia or analgesia. (See Labor and Delivery)"

This section should be revised using the multiple of the doses in animals and humans in term of AUC or body surface area unit. Accordingly, this section should read as follows:

"Teratogenicity studies in rats and rabbits did not show evidence of any adverse effects on organogenesis or early fetal development in rats (26 mg/kg) or rabbits (13 mg/kg). The doses used were approximately equal ——————————— dose based on body surface area. There were no treatment related effects on late fetal development, parturition, lactation, neonatal viability or growth of the offspring in 2 perinatal and postnatal studies in rats, at dose levels equivalent to the maximum recommended human dose based on body surface area. In another study at 23 mg/kg, an increased pup loss was seen during the first 3 days postpartum, which was considered secondary to impaired maternal care due to maternal toxicity.

There are no adequate and well-controlled studies in pregnant women of the effects of Naropin on the developing fetus. Naropin should only be used during pregnancy if the benefits outweigh the risk."

Recommendations

Sponsor has not requested any changes in the preclinical sections of the labeling and this reviewer did not find any important preclinical findings in the present submission that must be in the labeling. Therefore, this supplement NDA is approvable based on the standpoints of pharmacology. Before the supplement can be approved, however, amendment of the package insert is indicated. Please refer to Package Insert section for recommendations which should be transmitted to the Applicant.

Anwar Goheer

Concur by peer reviewer: Lucy Jean