CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER: NDA 20997

PHARMACOLOGY REVIEW(S)

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

NDA: 20-997 IND:

Submission: NDA Dated: April 27, 1998
Received by CDR: Apr. 27, 1998
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Review Completed: Nov. 6, 1998
Reviewer: M.A. Goheer, Ph.D.

Sponsor: Darwin Discovery Limited (changed from Chiroscience Limited)
Cambridge Science Park, Milton Road, Cambridge, CB4 4WE, England.

Sponsor's Authorized Representative: Parexel International Corporation, Waltham, MA.

Information to be conveyed to the sponsor: Yes

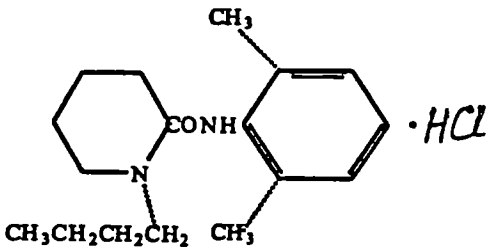
Drug Name: Chirocaine™ (levobupivacaine injection)

Strengths: 0.25%, 0.5%, and 0.75% (2.5, 5.0, and 7.5 mg/ml)

Other Names: (S)-bupivacaine HCL, (-)bupivacaine HCl:

Chemical Name: (S)-1-butyl-2-piperidylformo-2',6'-xylidide hydrochloride

Structure:



Molecular formula: $C_{18}H_{28}N_2O.HCl$ Molecular weight: 324.9
CAS Registry Number - 27262-48-2 Laboratory Code: D1249

Drug Manufacturer: Chiroscience R&D Limited, Wedgwood Way, Stevenage, Hertfordshire, SG 4QT, United Kingdom.

Dosage Form: White or almost white powder. Solution in 0.9% aqueous sodium chloride containing 2.5, 5.0 and 7.5 mg per ml of levobupivacaine as the HCl salt, is brought to volume in water for injection, pH 4.0-6.5. The solution is filtered, filled into ampules, sealed and sterilized. Chirocaine is preservative free and is available in 10 ml and 30 ml single dose vials.

Routes of Administration: Injection for local infiltration, peripheral nerve block and caudal and lumbar epidural blocks.

Indications and Dosages: Chirocaine (2.5, 5.0 and 7.5 mg/ml) is indicated for surgical anesthesia and pain management. The maximum dose administered as a postoperative epidural infusion over 24 hours was 570 mg (352 mg/m^2). Epidural doses of up to 375 mg (231 mg/m^2) have been administered incrementally to patients during a surgical procedure. The maximum dose administered to patients as a single fractionated injection was 300 mg (185 mg/m^2) for axillary brachial plexus blocks.

Pharmacologic Class: Amino amide class of local anesthetic.

Complete Formula:

Ingredient	Content		
	2.5 mg/ml	5.0 mg/ml	7.5 mg/ml
Levobupivacaine HCl	mg*	mg**	mg***
Sodium Chloride	mg	mg	mg
1 N Sodium Hydroxide	as required	as required	as required
1 N Hydrochloride Acid	as required	as required	as required
pH	4.0 - 6.5	4.0 - 6.5	4.0 - 6.5
Water for Injection	to 10.0 ml	to 10.0 ml	to 10.0 ml

* Equivalent to 25.0 mg of levobupivacaine base
** Equivalent to 50.0 mg of levobupivacaine base
*** Equivalent to 75.0 mg of levobupivacaine base

Related IND:

Related NDAs: 16-964 - Bupivacaine, 17-751 - Etidocaine, 20-533 - Naropin

Previously Reviewed Submissions: The following studies were reviewed under IND on July 3, 1997.

Ongoing Nonclinical Studies: The sponsor did do not submit the following acute pharmacodynamics studies in the NDA original submission because these studies are not completed.

Studies Reviewed Within This Submission:

NONCLINICAL PHARMACOLOGY AND TOXICOLOGY SECTION

Individual Nonclinical Study Reports

I Pharmacology Studies

1. Lee-Son, S. et al., Stereo selective Inhibition of Neuronal Sodium Channels by Local Anesthetics: Evidence for Two Sites of Action? (Anesthesiology, 1992; 77:324-35), vol. 1.11, p 109.

2. Dhyre, H. et al., The Duration of Action of Bupivacaine, Levobupivacaine, Ropivacaine and Pethidine in Peripheral Nerve Block. (Acta Anaesthesiol. Scand. 1997; 41:1346-1352), vol. 1.11, p 121.

5. Vanhoutte, F. et al., Stereo selective Effects of the Enantiomers of Bupivacaine on the Electrophysiological Properties of the Guinea-Pig Papillary Muscle (Br. J. Pharmacol. 1991; 103:1275-81), vol. 1.11, p 200.

8. Valenzuela, C. et al., Stereo selective Block of Cardiac Sodium Channels by Bupivacaine in Guinea-pig Ventricular Myocytes (Circulation. 1995a; 92:3014-24), vol. 1.11, p 285.

9. Valenzuela, C. et al., Stereo selective Block of a Human Cardiac Potassium Channel (Kv I.5) by Bupivacaine Enantiomers (Biophys J. 1995b; 69:418-27), vol. 1.11, p 293.

10. Graf, B.M. et al., Stereospecific Effect of Bupivacaine Isomers on Atrioventricular Conduction in the Isolated Perfused Guinea-pig Heart (Anesthesiology. 1997; 86:410-419), vol. 1.11, p 303.

11. Mazoit, J.X. et al., Myocardial Uptake of Bupivacaine: II. Pharmacokinetics and Pharmacodynamics of Bupivacaine Enantiomers in the Isolated Perfused Rabbit Heart. (Anesth. Analg. 1993; 77:477-82), vol. 1.11, p 313.

12. Denson, D.D. et al., Enantiomer-Specific Effects of an Intravenously Administered arrhythmogenic Dose of Bupivacaine on Neurones of the Nucleus Tractus Solitarius and the Cardiovascular System in the Anesthetized Rat (Regional Anesthesia.1992; 17:311-6), vol. 1.11, p 319.

II Toxicology Studies

1. Luduena, FP, Bogado, EF, Tullar, BF. Optical Isomers of Mepivacaine and Bupivacaine.

(Arch Int. Pharmacodyn. 1972; 200:359-69), vol. 1.13, p 001

2. Aberg, G. Toxicological and Local Anaesthetic Effects of Optically Active Isomers of Two Local Anaesthetic Compounds. (Acta pharmacol et toxicol. 1972; 31 :273-86), vol. 1.13, p 012.

III ADME Studies

6. Rutten, AJ, Mather, LE, McLean, CF. Cardiovascular Effects and Regional Clearances of I.V. Bupivacaine in Sheep: Enantiomeric Analysis. (Br. J Anaesth. 1991; 67:247-56), vol. 1.19, p 293.

7. Mather, LE. Dispositions of Mepivacaine and Bupivacaine Enantiomers in Sheep. (Br. J Anaesth. 1991; 67:239-46), vol. 1.19, p 303.

8. Rutten, AJ, Mather, LE, McLean, CF, Nancarrow, C. Tissue Distribution of Bupivacaine Enantiomers in Sheep (Chirality. 1993 5:485-91), vol. 1.19, p 311.

3.1 Method Validation Studies

IV. Carcinogenicity: None

V. Immunotoxicity: None

VI Reproductive Toxicology

[redacted]

VII. Genotoxicology:

VIII. Special Toxicology Studies: None

Studies not Reviewed Within This Submission: None

Note - Portions of this review were excerpted directly from the sponsor's submission.

I Pharmacology Studies

1. Lee-Son, S. et al., Stereo selective Inhibition of Neuronal Sodium Channels by Local Anesthetics: Evidence for Two Sites of Action? (Anesthesiology, 1992; 77:324-35), vol. 1.11, p 109. Four selective actions of four local anesthetics (bupivacaine, RAC 109, HS 38 and HS 37) were examined in isolated frog peripheral nerve and single sodium channels: tonic depression of compound action potentials at low stimulation frequency (one per minute); phasic depression of the compound action potential during trains of stimulation at 5, 10, and 20 HZ; competitive antagonism of the reversible Na channel activator veratridine assayed through the depolarization of the compound resting membrane potential; and depression of depolarization of the compound resting membrane potential initially induced by the irreversible channel activator batrachotoxin were assayed.

Based on the results from this study along with previous findings, local anesthetic molecules with more sharply angled shape have stronger stereoselectivities than fewer angled, more planar drugs.

2. Dhyre, H. et al., The Duration of Action of Bupivacaine, Levobupivacaine, Ropivacaine and Pethidine in Peripheral Nerve Block (Acta Anaesthesiol. Scand. 1997; 41:1346-1352), vol. 1.11, p 121.
Sprague-Dawley rats were subjected to infraorbital (IONB) or sciatic nerve block (SNB) by bupivacaine, levobupivacaine, ropivacaine or pethidine. The duration of a sensory block induced by equimolar doses of these agents was similar, although bupivacaine exerted more pronounced effect than levobupivacaine (AUC by 25%). Levobupivacaine and bupivacaine showed similar motor-blocking effects.

3. Neuropharmacological Investigations of Levobupivacaine: Actions on Peripheral Nerve Functions of the Rat in Vivo(

Report Date: Sept. 30, 1997
Author: G.R. Strichartz
Compounds: S(-)-bupivacaine, R(+)-bupivacaine and racemic bupivacaine
Certificate of Analysis: the assay by % w/w pure
Content of enantiomer (R) - 0.1% w/w.
Both bupivacaine stereo isomers and the racemic mixture produced a reversible nerve block in the rats.

4. Neuropharmacological Investigations of Levobupivacaine: Actions on Peripheral Nerve Functions and Cutaneous Nociception of the Rat In Vivo

Report Date: Feb. 27, 1998
Report No.: II
Author: G.R. Strichartz
Compounds: S(-)-bupivacaine, R(+)-bupivacaine and racemic bupivacaine.
Strain: Male Sprague-Dawley rats, 220-250 g body weight.
Inhibition of sciatic nerve-mediated functions (motor, proprioceptive and nociceptive: pinch and heat stimulation of the ipsilateral foot) were assessed by semi-quantitative neurological tests. Motor and proprioceptive functions were more sensitive than nociceptive functions for all three drugs. All three drugs tested produced equipotent motor and proprioceptive effects. Infiltrations of the skin at higher concentration (0.075%) of three drugs were virtually equipotent, producing 90-100% inhibition of nocifensive response and lasting for the same period. The full recovery occurred at approximately two hours after injection.

5. Vanhoutte, F. et al., Stereo selective Effects of the Enantiomers of Bupivacaine on the Electrophysiological Properties of the Guinea-Pig Papillary Muscle (Br. J. Pharmacol. 1991; 103:1275-81), vol. 1.11, p 200. Direct myocardial effects of the S(-) and R(+) bupivacaine were compared in the guinea-pig isolated papillary muscle. The S(-) bupivacaine affects the maximal rate of depolarization (V_{max}) and action potential duration (APD) less than the R(+) enantiomer at different rates of stimulation and resting membrane potentials.

6. Contractile Effects of Levobupivacaine, Bupivacaine (Marcaine) and Ropivacaine (Naropin): Studies in Human (Atrial) and Guinea-pig (Ventricular) Cardiac Muscle (Study number D1249-024-PH, D. Harding), vol. 1.11, p 207.
Cells from the hearts of male Dunkin-Hartley guinea-pig (250-350 g) and samples of a right atrial appendage from patients undergoing a coronary artery bypass or aortic valve replacement surgery were used. Levobupivacaine, bupivacaine and ropivacaine produced a 50% decrease (CI-50) in peak shortening amplitude at 12.9±2.3, 10.3±0.6 and 18.0±2.6 µM, respectively, in guinea-pig left ventricular myocytes. Similar effects on contraction were found in guinea-pig right ventricular papillary muscles. There were no significant differences in the decrease in developed tension between three local anesthetics at either 3 µM or 30 µM in human pectinate muscles. In human myocardium, mean developed force decreased by 37% at 3 µM and 75% at 30 µM for the three local anesthetics.

7. The Electrophysiological Effects of Levobupivacaine, Bupivacaine (Marcain) and Ropivacaine (Naropin): Studies on the Action Potential and Developed Force of Guinea-pig Isolated Ventricular Cardiac Muscle
247.
Action potential amplitude (APA), action potential duration to 90% repolarisation (APD_{90}), and maximum rates of rise of membrane potential (V_{max}) were measured in papillary muscles. All three anesthetics produced a similar, marked decrease in developed force at both 3 and 30 µM. The developed force recovery was faster for levobupivacaine compared with bupivacaine. Both levobupivacaine and ropivacaine produced less decrease in V_{max} than bupivacaine at 30 µM concentration. Levobupivacaine, bupivacaine and ropivacaine at 30 µM produced a significant reduction in APA compared to control but no difference between drug groups. APA and APD_{90} recovered fully after 40 minute washouts for all 3 drugs.

8. Valenzuela, C. et al., Stereo selective Block of Cardiac Sodium Channels by Bupivacaine in Guinea-pig Ventricular Myocytes, (Circulation. 1995a; 92:3014-24), vol. 1.11, p 285.
R(+)- and S(-)-bupivacaine produced similarly but limited levels of tonic blocks in isolated guinea pig ventricular myocytes. During long depolarization (5 seconds at 0 mV), R(+)-bupivacaine induced a larger inhibitory effect on the cardiac sodium current (I_{Na}: 72±2% versus 58±3% for the S(-)-enantiomer). The voltage dependence of the availability of the Na^+ current was shifted to more hyperpolarizing potentials compared with the control. The apparent affinities of the activated or open state for S(-)- and R(+)-bupivacaine was 4.3 and 3.3 µmol/L, respectively.

9. Valenzuela, C. et al., Stereo selective Block of a Human Cardiac Potassium Channel (Kv I.5) by Bupivacaine Enantiomers, (Biophys J. 1995b; 69:418-27), vol. 1.11, p 293.
The main conclusions from the interaction between S(-)- and R(+)-bupivacaine on a cloned human cardiac potassium channel are as follows: 1) the charged form of both bupivacaine enantiomers blocks the hkv1.5 channel after it opens, 2) unbinding is required before the channel can close, 3) binding occurs within the transmembrane electrical field, and 4) block of hkv1.5 by bupivacaine is markedly stereo selective, with the R(+)-enantiomer being more potent.

10. Graf, B.M. et al., Stereospecific Effect of Bupivacaine Isomers on Atrioventricular Conduction in the Isolated Perfused Guinea-pig Heart(Anesthesiology. 1997; 86:410-419), vol. 1.11, p 303.
The hearts of 12 guinea pigs were perfused with increasing randomized concentrations of racemate and both isomers of bupivacaine; the results are shown on the next page.

	Atrial Heart Rate (beats/min)			Coronary Flow (ml/g/min)			Oxygen Extraction (%)		
	Racemate	(+)Isomer	(-)Isomer	Racemate	(+)Isomer	(-)Isomer	Racemate	(+)Isomer	(-)Isomer
Control 1	227±4	227±5	226±5	6.9±0.6	6.9±0.7	6.6±0.4	68±4	67±6	68±4
0.5 µM	222±2	223±6	222±6	6.2±0.5	6.4±1.0	6.3±0.4	65±6	64±6	65±4
1 µM	216±2	219±7	216±6	6.0±0.5	6.2±0.9	6.0±0.6	64±7	63±6	63±5
5 µM	202±4	206±8	205±5	5.4±0.5	5.5±0.8	5.4±0.6	61±8	62±4	62±6
10 µM	187±3	191±8	188±6	5.0±0.8	5.1±0.8	4.9±0.5	59±7	59±6	60±6
Control 2	228±6	230±7	229±4	7.8±0.8	7.5±0.8	7.7±0.9	65±5	65±3	66±5

Racemic and isomeric bupivacaine equally and doses dependently decreased cardiac function. The (+)isomer significantly prolonged atrioventricular (AV) conduction time compared with the racemate and the (-)-isomers at all concentrations. The greater delay in AV time with the (+) than the (-) or racemate led to a second-degree AV dissociation in 10 of 12 hearts treated with (+)-bupivacaine.

11. Mazoit, J.X. et al., Myocardial Uptake of Bupivacaine: II. Pharmacokinetics and Pharmacodynamics of Bupivacaine Enantiomers in the Isolated Perfused Rabbit Heart, (Anesth. Analg. 1993; 77:477-82), vol. 1.11, p 313.
The racemic and two isomers of bupivacaine showed similar myocardial pharmacokinetics in the isolated perfused rabbit heart. The tissue/perfusate concentration ratio at steady state was similar for the three drugs. The QRS widening and severe arrhythmias were less pronounced in the hearts receiving the S(-) isomer than in the hearts receiving the racemic mixture or R(+)isomer.

12. Denson, D.D. et al., Enantiomer-Specific Effects of an Intravenously Administered arrhythmogenic Dose of Bupivacaine on Neurones of the Nucleus Tractus Solitarius and the Cardiovascular System in the Anesthetized Rat, (Regional Anesthesia.1992; 17:311-6), vol. 1.11, p 319.
The cell firing rates decreased in adult Sprague-Dawley rats after the injection of 2 mg/kg of either d- or l-bupivacaine. Besides decrease in blood pressure, mild bradycardia (4/24) was noted in animals receiving l-bupivacaine. d(+)-Bupivacaine produced severe bradycardia, hypotension and death in all animals. All l(-)-bupivacaine treated animals continued to breathe and all but two of the animals survived.

13. Pharmacological and Pharmacokinetic Studies with Intravenous Levobupivacaine and Bupivacaine in Sheep

Compound: Levobupivacaine and bupivacaine

Dose Levels:
Lower dose range/animal - 12.5, 25 and 37.5 mg bupivacaine
6.25, 12.5, 18.75, 25, and 37.5 mg levobupivacaine
Higher dose range/animal - 75, 100, 150, and 200 mg bupivacaine
37.5, 50, 100, 150, and 200 mg levobupivacaine
Strain: Adult Merino ewes (mean body weight 51 kg)
Number: Two groups of 7 animals
Route: Intravenous infusion - one minute for the lower dose
Three minutes for higher dose
Study Site: Centre for Anaesthesia and Pain Management Research,
Department of Anaesthesia and Pain Management,
The University of Sydney at Royal North Shore Hospital, St. Leonards, NSW 2065, Australia.
Study Director: Prof. L.E. Mather
GLP/QAU: The study procedures were approved by the Animal Care and Ethics Committee of the Royal North Shore Hospital.

The drugs were administered in conscious previously instrumented sheep in random order and at least 48 hours were allowed between experiments in the same sheep. Parameters were recorded up to 30 minutes (a lower dose regimen) or 60 minutes (higher dose regimens) after the start of infusion.

Results: CNS, Inotropic and Hemodynamic Effects.

Mortality: All animals survived all doses of levobupivacaine (83 doses). Three animals died of the administration of bupivacaine at doses of 200, 150 and 150 mg per animal.
Central nervous system: Bupivacaine ≥75 mg and levobupivacaine ≥100 mg caused convulsive behavior. A convulsive behavior duration ratio also increased in a dose dependent manner, with bupivacaine producing longer duration than equal doses of levobupivacaine.
Inotropic effects as determined by left ventricular dP/dt_{max} and myocardial segmental length: There were significant time and dose-related decreases in dP/dt_{max} but no significant differences in magnitude of effect from the same dose of each drug. Doses of ≥75 mg bupivacaine or ≥100 mg levobupivacaine produced a biphasic effect on dP/dt_{max}. Higher doses also affected the time courses of the end-diastolic and end-systolic myocardial segment lengths.
Mean arterial pressure (MAP): Higher doses increased MAP.
Cardiac output (CO): There were no effects on CO at lower doses of both drugs. Higher doses sometimes decreased CO followed by increase; the predominant effects were net decrease in CO.
Left coronary blood flow (LCBF): Decreased LCBF at higher doses (≥100 mg).
Brain blood flow index (BBF): Bupivacaine at 75 and 100 mg produced net decrease in BBF and markedly deleterious effects at higher doses.
Levobupivacaine at 150 and 200 mg produced decrease in BBF that recovered more than 60 minutes.
Relevant acid-base changes: No systematic dose- or time-related differences between levobupivacaine and bupivacaine.
Electrocardiological analysis: Bupivacaine and levobupivacaine produced a similar increase in QRS duration and shortened QTC intervals.

Bupivacaine at ≥75 mg and levobupivacaine at ≥100 mg increased HR.
Bupivacaine and levobupivacaine at 75 mg induced sinus tachycardia in 6/6 and 0/6 animals, respectively.
Bupivacaine (150 mg & 200 mg) induced higher numbers and longer duration of VA than levobupivacaine.

Systemic and regional pharmacokinetics: Pharmacokinetic properties, determined by reference to three compartment open model.

Drug Dose (mg)	Enan-tiomer	n	initial dilution volume (L)	Total distribution volume (Vss) (L)	Total body clearance (CL) (L/min)	Terminal half life (T1/2β) (min)
Bupivacaine all doses (75 - 200)	R-	19	2.9±0.7	56±26	1.56±0.84	40±15
	S-	19	2.8±0.8	63±39	1.10±0.76	59±30
Levobupivacaine all dose (37.5 - 200)		33	2.7±0.9	66±42	1.16±0.74	63±32

The concentrations of S-bupivacaine usually exceed R-bupivacaine after administration of bupivacaine. The concentrations of S-bupivacaine after bupivacaine administration were equal to those of levobupivacaine for the same dose.

14. Effects of High Doses of Levobupivacaine on the Cardiovascular and Central Nervous Systems of the Sheep.

Compound: Levobupivacaine
Dose Levels: 200, 250, 300, and 350 mg
Strain: Adult Merino ewes (mean body weight 50 kg)
Number: Two groups of seven animals
Route: Intravenous infusion - in more than three minutes
Study Site: Centre for Anaesthesia and Pain Management Research,
Department of Anaesthesia and Pain Management,
The University of Sydney at Royal North Shore Hospital, St. Leonards, NSW 2065, Australia.
Study Director: Prof. L.E. Mather
Study Sponsor: Chiroscience Ltd.
GLP/QAU: The study procedures were approved by the Animal Care and Ethics Committee of the Royal North Shore Hospital.

The drugs were administered in conscious previously instrumented sheep in random order and at least 24 hours were allowed between experiments in the same sheep. Mean arterial blood pressure (MABP), heart rate (HR), cardiac output (CO), left coronary artery blood flow (CABF), sonomicrometry-measured segmental shortening (SS), and the electrocardiogram (ECG) measurements were recorded to 60 minutes after the start of infusion. The blood

samples were taken for pharmacokinetic analysis.

Results: CNS, Inotropic and Hemodynamic Effects.

Mortality:

Weight	Doses administered (mg)	Fatal dose (mg)
49	250	250
46	250	250
43	250, 300	300
55	200, 250, 300	300
47	250, 300	300
53	200, 250, 250, 300	300
58	250, 300, 350	350
52	250, 300, 350	350

Death was related to cardiac intoxication every time.

Central nervous system: All doses produced convulsive behavior. The duration of convulsive behavior was not dose related and dissociating the direct cardiac effect from those induced by central mechanisms was difficult.

Electrocardiological effects: An increase in QRS width (30-40%) in all sheep at every dose. Arrhythmias (supraventricular, ventricular, bigeminy, trigeminy) occurred at all doses in all animals

Cardiovascular and hemodynamic effects:

Heart Rate: Increased heart rates associated with CNS excitation

Mean arterial blood pressure (MABP): Higher with the onset of CNS excitation

Cardiac output (CO): Not obtained for all animals, initially decreased CO followed by increase; the predominant effects were net decrease in CO.

Left ventricular segment shortening (SS): No reliable measurements but decreased abruptly with the onset of fetal arrhythmias.

Left coronary blood flow (LCBF): variable results when available.

Fatal doses:

Dose rate (mg)	Cause of death	Time of death (min)	Mean time of death (min)
250	EMD/fill failure	12.5	
250	failure to fill (rate >600)	29.0	20.8±11.7
300	EMD	4.8	
300	VF	2.1	
300	VF	2.3	
300	Fill failure/VT	12.8	5.5±5.0
350	VF	2.8	
350	EMD	3.8	3.3±0.7

EMD - electromechanical dissociation
VF - ventricular fibrillation
VT - ventricular tachycardia

Effects on acid-base balance: Convulsive behavior induced transitory increases in arterial blood pH followed by acidosis due to lactic acid burden from muscular activity. There was no extended change of blood pH from any dose in any animal.

Pharmacokinetic analysis:

Dose (mg)	Initial dilution volume (L)	Total distribution volume (L)	Total clearance (L/min)	Terminal half life (min)
250	3.6	1.25	131	
200	3.8	1.90	102	51
250	4.7	1.7	65	40
250	2.8	1.24	101	90
250	4.2	2.17	102	48
300	6.2	2.53	98	115
250	7.2	1.41	131	85
300	1.6	1.48	75	54
200	5.2	1.62	80	48
250	5.4	1.72	82	51
All doses	4.5±1.6	1.7±0.4	97±22	70±29

15. The Relative Myocardial Toxicity of Bupivacaine, Levobupivacaine and Ropivacaine: An Electrophysiological Study Using Intra coronary Injection of Local Anaesthetics in the Pig.

Compounds: Racemic bupivacaine supplied by
Levobupivacaine supplied by (99.4% w/w enantiomeric purity) and levoropivacaine supplied by

Route of Administration: Left anterior descending coronary artery
Animals: Farm-bred female pigs (44.9±5.4 kg)
Supplier:
Study Director: Sebastian Reiz, MD, Ph.D.,
Professor and Chairman, Department of Anesthesiology,
Centre Hospitalier Universitaire Vaudois, CH-1011 Lausanne, Switzerland
Study Sponsor: Chiroscience Ltd., Cambridge Science Park,
Milton Road, Cambridge CB4 4WE, UK.
GLP Statement: Yes

An angiography catheter was used for the direct administration of local anesthetics into the left anterior descending coronary artery. This method excluded electrophysiological effects mediated by central mechanisms. In this study, the sponsor compared the electrophysiological effect of Intra coronary racemic bupivacaine, levobupivacaine and ropivacaine in ventilated swine.

Results:

Homeostasis: At the end of stabilization period.

Compound	pH	P_aO_2 (mm Hg)	P_aCO_2 (mm Hg)	Hct (%)	Temp (°C)
Bupivacaine (n=7)	7.52± 0.07	210± 59	33± 7	27± 3	38.4± 0.5
Levobupivacaine (n=7)	7.49± 0.07	190± 52	31± 5	26± 3	38.3± 1.0
Ropivacaine (n=6)	7.49± 0.03	186± 49	37± 1	27± 3	39.0± 0.9

Doses injected with mode of death. The lethal doses for the three agents were:
Bupivacaine - 5.0±0.8 mg (4 - 6 mg)
Levobupivacaine - 7.9±0.9 mg (7 - 9 mg)
Ropivacaine - 8.2±2.4 mg (4 - 10 mg)
All animals except two (one in group R and one in group B) died of ventricular fibrillation.

Electrophysiological Toxicity. Baseline values for the QRS, QTC and PQ intervals were similar among the three groups.

Changes in QRS (msec)

Control	Compound	0.375 (mg)	0.75 (mg)	1.5 (mg)	3 (mg)	4 (mg)	5 (mg)	6 (mg)	7 (mg)	8 (mg)	9 (mg)
61.6 ±4.0 (n=7)	Bupivacaine	-0.19 ±1.0 (n=7)	2.2 ±3.6 (n=7)	10.3 ±10.5 (n=7)	36.1 ±21.4 (n=7)	47.8 ±24.4 (n=5)	90.7 ±17.9 (n=2)				
63.0 ±3.8 (n=7)	Levo-bupivacaine	1.4 ±2.1 (n=7)	3.8 ±4.6 (n=7)	7.9 ±7.9 (n=7)	18.3 ±12.5 (n=7)	35.8 ±17.3 (n=7)	59.9 ±15.0 (n=7)	71.1 ±24.5 (N=7)	85.1 ±28.5 (n=4)	127.8 ±40.7 (n=2)	
62.5 ±4.3 (n=6)	Ropivacaine	-0.4 ±1.4 (n=6)	0.0 ±2.8 (n=6)	6.9 ±5.7 (n=6)	20.4 ±15.1 (n=6)	32.1 ±31.6 (n=5)	40.7 ±32.5 (n=5)	51.8 ±32.1 (n=5)	48.6 ±24.3 (n=4)	59.8 ±19.4 (n=3)	59.1 ±4 (n=3)

Changes in QTC (msec)

Control	Compound	0.375 (mg)	0.75 (mg)	1.5 (mg)	3 (mg)	4 (mg)	5 (mg)	6 (mg)	7 (mg)	8 (mg)	9 (mg)
441 ±32 (n=7)	Bupivacaine	-0.1 ±5.2 (n=7)	-1.1 ±7.4 (n=7)	0.4 ±9.1 (n=7)	2.2 ±15.0 (n=7)	22.6 ±26.4 (n=5)	61.8 ±16.6 (n=2)				
453 ±26 (n=7)	Levo-bupivacaine	0.5 ±4.6 (n=7)	2.0 ±7.2 (n=7)	5.5 ±10.5 (n=7)	11.0 ±13.6 (n=7)	31.3 ±26.2 (n=7)	58.4 ±35.9 (n=7)	57.9 ±43.7 (N=7)	73.2 ±31.3 (n=4)	80.6 ±1.4 (n=2)	
461 ±21 (n=6)	Ropivacaine	-0.5 ±3.4 (n=6)	3.0 ±9.9 (n=6)	7.7 ±15.0 (n=6)	11.6 ±21.0 (n=6)	18.1 ±30.3 (n=5)	12.8 ±29.9 (n=5)	26.3 ±39.2 (n=5)	27.6 ±13.5 (n=4)	49.3 ±30.8 (n=3)	57.9 ±38.1 (n=3)

Changes in PQ (msec)

Control	Compound	0.375 (mg)	0.75 (mg)	1.5 (mg)	3 (mg)	4 (mg)	5 (mg)	6 (mg)	7 (mg)	8 (mg)	9 (mg)
102 ±9 (n=7)	Bupivacaine	-2.3 ±3.9 (n=7)	-2.7 ±3.9 (n=7)	-1.5 ±3.7 (n=7)	1.1 ±3.9 (n=7)	0.4 ±3.3 (n=5)	-2.3 ±7.1 (n=2)				
103 ±7 (n=7)	Levo-bupivacaine	1.0 ±1.5 (n=7)	1.1 ±1.3 (n=7)	1.1 ±1.7 (n=7)	1.2 ±2.8 (n=7)	3.0 ±3.0 (n=7)	1.8 ±3.8 (n=7)	3.8 ±4.4 (N=7)	0.8 ±5.1 (n=4)	0.6 ±7.6 (n=2)	
102 ±5 (n=6)	Ropivacaine	1.9 ±2.8 (n=6)	2.0 ±1.6 (n=6)	3.6 ±3.0 (n=6)	3.3 ±3.4 (n=6)	4.8 ±3.4 (n=5)	5.6 ±4.8 (n=5)	8.1 ±7.5 (n=5)	8.3 ±7.9 (n=4)	8.9 ±5.8 (n=3)	10.0 ±6.7 (n=3)

Excluded Animals: Six animals were excluded from the study due to right coronary artery catheterization because of mistaken inversion of the fluoroscopic images.

II Toxicology Studies

1. Luduena, FP, Bogado, EF, Tullar, BF. Optical Isomers of Mepivacaine and Bupivacaine. (Arch int Pharmacodyn. 1972; 200:359-69), vol. 1.13, p 001. Intra spinally in rabbits, the (-)-isomer of bupivacaine was 40% more active than its enantiomer. The spinal anesthesia produced in rabbits by (-)-bupivacaine was of longer duration than that of the (+) form. The intraderm irritancy of the racemic (+)- and (-) forms were the same. (-)-Bupivacaine is less toxic than the racemic and the (+)-forms by intravenous injections in mice, rats and rabbits and by subcutaneous injection in rats and mice.

2. Aberg, G. Toxicological and Local Anaesthetic Effects of Optically Active Isomers of Two Local Anaesthetic Compounds (Acta pharmacol et toxicol. 1972; 31 :273-86), vol. 1.13, p 012. D (+)-isomer of bupivacaine was more toxic than the racemic when given intravenously or subcutaneously in mice and rats.

3. Bupivacaine HCl and S-Bupivacaine HCl: Acute Intravenous Toxicity (LD50) Test in Rats Reviewed on July 3, 1997 under IND

The median intravenous lethal doses (LD_{50}s) were estimated at 4.35 mg/kg for bupivacaine and 4.97 mg/kg for S-bupivacaine.

4. Bupivacaine HCl & S-Bupivacaine HCl: Study for Comparative Effects of a Single Epidural Dose in Dogs Reviewed on July 3, 1997 under IND

5. Bupivacaine HCl & S-Bupivacaine HCl: Toxicity to Dogs by Repeated (Once Weekly) Epidural Administration for 3 Weeks . Reviewed on July 3, 1997 under IND

Dose Levels: 0.2 ml/kg of a 0.75% solution

Results

1. Clinical Observations: ~~Total loss of motor function in the hindquarters~~ to unsteadiness Total loss of motor function in the hindquarters to unsteadiness of the gait. Recovery started two hours after dosing and in some animals were normal the following day.
2. Mortality: None
3. Bodyweight: No effect
4. Food Consumption: No effect of treatment.
5. Neurological Examination: No difference
6. Organ Weights: No difference
7. Macroscopic Examination: There was no evidence of an effect of treatment.
8. Microscopic Examination: No effect of treatment on lumbosacral region of spinal cord with meninges, lumbosacral dorsal root ganglia and nerve roots, cauda equina and sciatic nerves.

6. Levobupivacaine single dose intrathecal tolerance study in the Cynomolgus monkey

Report for: Chiroscience Ltd., Cambridge Science Park, Milton Road, Cambridge CB4 4WE, England
Author: Dr. rer. Nat. Ulrich Zuhlke
Test Facility:

Report No. 1406-1193-133
Report Issue: Sept. 26, 1997
GLP/QA Statements: Yes
Animals: Cynomolgus monkeys 3.7 - 7.1 kg b.w., 2 - 8 years old.
Supplier:
Test Article: Levobupivacaine HCL., Batch No. 22801
Positive Control: Bupivacaine HCl. , Batch No. 32203
Route of Administration: Intrathecal by bolus injection (lumbar area).

Dose Groups:

Group Number	Group	Dose Level (mg/ml)	Dose Volume (ml/dose)	Dose (mg/kg)	Animals/ group
1	Control	0	0.6	0.0	5
2	Positive Control	5	0.3	0.3	5
3	Low	5	0.3	0.29	5
4	High	5	0.6	0.58	5

Termination: Eight days after dosing

Results

Mortality: None
Clinical Observations: Transient slightly decreased motor activity in group 2 (4/5 animals), group 3 (3/5 animals) and group 4 (3/5 animals).
Body Weight: No effect
Food Consumption: No difference
Neurological Examination: Decreased motor activity and ataxia.
Organ Weights: No apparent treatment related changes.
Macroscopic Necropsy Findings: No treatment related changes
Microscopic Necropsy Findings: No effects on brain and spinal cord sections.

III ADME Studies

1. Pharmacokinetics after a single dose of [^{14}C]levobupivacaine HCL: disposition in the rat following intravenous administration (IRI 159365), vol. 1.18, p 001.

Sponsor: Chiroscience Limited, Milton Road, Cambridge, England.
Performing Laboratory:
Project No.: 159365
GLP Statement: Yes
Experimental Completion Date: Feb. 23, 1997
Compounds: [^{14}C]-Levobupivacaine (Batch No. CFQ9713)
Non-radiolabelled levobupivacaine (Batch No. 22801)
Animals: 8 male albino Sprague Dawley rats (age 7-8 weeks, 296 - 312 g body weight)
6 male pigmented PVG rats (age 7 - 8 weeks, body weight 194 - 218 g)
Route: Intravenous by tail vein

Results

Cumulative Recovery of total radioactivity following single intravenous administration of [14C]-

levobupivacaine to rats. Results expressed as % administered doses.

Time (h)	Urine	Feces	Cage washes	Recovery
6	5.9±1.3	-	-	5.9±1.3
24	17.6±3.0	42.4±11.4	2.4±2.5	62.4±6.8
48	20.2±2.0	62.2±7.2	2.8±2.7	85.6±3.5
72	21.0±2.4	67.4±5.8	2.9±2.7	91.4±1.9
96	21.4±2.5	69.2±6.4	3.1±2.6	93.7±1.8
120	21.5±2.6	69.8±6.4	3.1±2.6	94.5±1.8
168	21.7±2.6	70.4±6.3	3.2±2.6	95.8±1.7

Highest concentration of total radioactivity in the plasma was 0.55 μg equiv.ml at 5 min post dose.

2. The Disposition of [^{14}C]-bupivacaine and [^{14}C]-levobupivacaine following intravenous administration to male F344 rats

Sponsor: Chiroscience Plc. , Milton Road, Cambridge, England.
Performing Laboratory:

Study Completion Date: Jan. 26, 1998
Animals: Male F344 rats (220 - 250 g body weight)
Compounds: [^{14}C]-bupivacaine and [^{14}C]-levobupivacaine were supplied by

Results

Cumulative recovery of total radioactivity from male F344 rats dosed intravenously with either 1 mg [^{14}C]-bupivacaine/kg body weight or 1 mg [^{14}C]-levobupivacaine/kg body weight.

Time (h)	Urine		Feces		Total Excretion	
	Bupi-vacaine	Levobupi-vacaine	Bupi-vacaine	Levobupi-vacaine	Bupi-vacaine	Levobupi-vacaine
0 - 24	29.2±1.9	28.1±3.0	5.4±7.3	2.3±2.6	34.6±7.2	30.4±3.4
24 - 48	39.3±1.7	38.3±3.6	19.8±4.5	19.8±5.9	59.1±3.1	58.1±8.3
48 - 72	44.3±2.0	44.3±2.3	37.4±8.0	32.8±8.3	81.7±8.8	77.1±8.6
72 - 96	46.9±3.0	46.9±1.9	46.8±3.9	43.4±5.7	93.7±2.5	90.3±3.9
96 - 120	47.8±3.2	47.7±2.3	48.0±4.2	44.7±5.3	95.8±2.6	92.4±3.0

Pharmacokinetic parameters of bupivacaine, levobupivacaine and dextrobupivacaine after intravenous administration to male F344 rats (1 mg/kg body weight).

Pharmacokinetic parameter	Bupivacaine	Levobupivacaine	Dextrobupivacaine
C_{max} (μg/ml)	0.33±0.05	1.47±0.57	0.92±0.47
$T_{1/2}$ (min)	21.8±12.6	11.0±4.4	10.1±4.8
AUC∞ (μg.min/ml)	34.2±1.2	67.5±13.6	49.9±13.4
Cl (ml/min)	6.5±0.2	3.6±0.6	4.9±1.1
$T_{1/2}$ β (hr)	11.1±1.8	6.3±2.0	7.0±2.2

3. Pharmacokinetics after a single dose of [^{14}C] bupivacaine HCL: disposition in the rat following subcutaneous administration (IRI 159386), vol. 1.18, p 081.

Sponsor: Chiroscience Limited, Milton Road, Cambridge, CB4 4WE, England.
Performing Laboratory:
Project No.: 159386
GLP/QA Statements: Yes
Study Completion Date: Sept. 29, 1997
Route: Subcutaneous administration
Compounds: [14C]-bupivacaine, batch no. CFQ9714
Non-radiolabelled bupivacaine, batch no. 32203
Dose: 10 mg/kg, single dose
Animals: Male and female Sprague Dawley rats (7 - 8 weeks old, 242 - 298 g body weight)

Results

Cumulative recovery of total radioactivity in male and female rats.

Time (h)		Urine	Feces	Cage Wash	Recovery
6	M	11.1±2.6	-	-	11.1±2.6
	F	17.2±1.3	-	-	17.2±1.3
24	M	27.4±4.2	45.9±11.0	1.3±0.6	74.6±6.9
	F	42.8±5.1	29.3±2.1	1.6±1.2	73.6±8.3
48	M	30.0±4.7	59.8±7.5	1.7±0.3	91.5±3.5
	F	47.5±3.4	41.8±2.7	2.1±1.1	91.4±2.1
72	M	30.4±4.8	62.1±6.6	1.8±0.3	94.4±2.4
	F	48.2±3.2	44.3±4.1	2.2±1.1	94.7±2.0
96	M	30.6±4.8	62.8±6.6	1.9±0.3	95.3±2.7
	F	48.5±3.3	44.7±4.2	2.3±1.1	95.5±2.0
120	M	30.7±4.8	63.2±6.5	2.0±0.3	95.8±2.6
	F	48.6±3.3	44.9±4.2	2.2±1.1	95.9±1.9
168	M	30.7±4.9	63.4±6.6	2.1±0.3	96.9±2.4
	F	48.7±3.3	45.2±4.2	2.6±1.2	97.1±1.8

Peak mean plasma concentrations of radioactivity in male and female rats were 1.49 µg equiv./ml at 0.75 h and 1.31 µg equiv./ml at 2 h after dosing, respectively.

4. Pharmacokinetics after a single dose of [^{14}C]-levobupivacaine HCL: disposition in the rat following subcutaneous administration (IRI-159370), vol. 1.18, p 142.

Sponsor: Chiroscience Limited, Milton Road, Cambridge, CB4 4WE, England.
Performing Laboratory: [redacted]
Project No.: 159370
GLP/QA Statements: Yes
Study Completion Date: Sept. 29, 1997
Route: Subcutaneous administration at the back of the neck.
Compounds: [14C]-levobupivacaine, batch no. CFQ9713
Non-radiolabelled bupivacaine, batch no. 22801
Dose: 10 mg/kg, single dose
Animals: Male and female Sprague Dawley rats (8 - 10 weeks old, 223 - 326 g body weight)

Results

Cumulative recovery of total radioactivity in male and female rats.

Time (h)		Urine	Feces	Cage Wash	Recovery
6	M	4.2±2.7	-	-	4.2±2.7
	F	9.2±10.0	-	-	9.2±10.0
24	M	17.4±2.1	42.9±9.5	1.9±0.2	62.3±8.0
	F	31.8±16.5	38.3±6.1	1.4±0.4	71.5±14.7
48	M	20.4±2.7	57.2±12.4	2.4±0.4	80.0±10.1
	F	35.1±15.9	53.2±14.6	1.8±0.5	90.1±3.1
72	M	21.4±3.3	66.2±5.7	2.7±0.2	90.3±2.6
	F	36.0±15.7	56.5±14.8	1.9±0.6	94.5±1.2
96	M	21.6±3.4	68.4±3.8	3.0±0.3	93.0±0.6
	F	36.2±15.8	57.2±15.0	2.0±0.5	95.3±0.9
120	M	21.7±3.4	68.9±3.5	3.0±0.2	93.6±0.7
	F	36.2±15.8	57.3±15.0	2.0±0.5	95.6±0.9
168	M	21.8±3.4	69.3±3.5	3.4±0.3	95.0±0.2
	F	36.3±15.8	57.5±15.1	2.1±0.4	96.4±1.0

Peak mean plasma concentrations of radioactivity in the female and male rats were 0.72 µg equiv./ml at 0.5 h post dose and 1.1 µg equiv./ml at 0.75 h post dose, respectively. Mean plasma concentration then continued to decrease. At 2 h post dose, the radioactivity was widely distributed in tissues with highest concentration in harderian, preputial, and adrenal glands. By 8 hours post dose, highest concentrations of radioactivity were measured in liver, adrenal gland and large intestinal wall.

5. The Disposition of [^{14}C]-levobupivacaine in the Rabbit Following Subcutaneous Administration (IRI 159391), vol. 1.18, p 200.
Sponsor: Chiroscience Limited, Milton Road, Cambridge, CB4 4WE, England.
Performing Laboratory:
Project No.: 159391
GLP/QA Statements: Yes
Study Completion Date: Sept. 3, 1997
Route: Subcutaneous administration at the back of the neck.
Compounds: [^{14}C]-levobupivacaine, batch no. CFQ9713
Non-radiolabelled bupivacaine, batch no. 22801

Dose: 5 mg/kg, single dose
Animals: Six female New Zealand White rabbits (3 months old, 2.3 - 3.1 kg body weight)
Supplier:

Results

Time (h)	Urine	Feces	Cage wash	Recovery
6	6.9	-	-	6.9
24	66.4±4.6	3.3±0.2	10.9±2.4	80.6±2.0
48	68.2±5.2	4.7±0.5	12.1±2.7	85.0±4.1
72	72.9±5.3	5.1±0.5	13.1±2.4	91.1±2.7
96	73.0±5.3	5.30.5	13.3±2.5	91.6±2.7
120	73.2±5.3	5.5±0.4	13.4±2.5	92.2±2.7
168	73.5±5.1	5.8±0.3	14.0±2.6	0.03
0.64	0.28	-	-	-

Peak mean plasma concentration was 1.1 μg equiv./ml at 1 hour post dose. analysis of urine showed three major radiolabelled components.

6. Rutten, AJ, Mather, LE, McLean, CF. Cardiovascular Effects and Regional Clearances of I.V. Bupivacaine in Sheep: Enantiomeric Analysis. (Br J Anaesth. 1991; 67:247-56), vol. 1.19, p 293. Racemic (RS)-bupivacaine hydrochloride 1 mg/min was infused in five sheep. Cardiovascular effects and the regional and total body clearances were determined at two steady state periods.

Mean arterial blood concentrations (mg/liter) of bupivacaine enantiomers at 3-4 h and 23-24 h.

Sheep No.	3-4 h R(+)-	S(-)-	R:S Ratio	23-24 h R(+)-	S(-)-	R:S Ratio
1	0.58±0.07	0.88±0.10	0.66	NA	NA	NA
2	0.20±0.01	0.22±0.01	0.91	0.24±0.01	0.27±0.01	0.89
3	0.25±0.03	0.39±0.03	0.64	0.28±0.01	0.42±0.02	0.67
4	0.52±0.04	0.75±0.06	0.69	0.53±0.01	0.54±0.03	0.99
5	0.66±0.04	0.94±0.03	0.70	0.68±0.03	0.83±0.07	0.82

There was no significant clearance of either enantiomer by the lungs, brain, heart, gut, kidneys or hindquarters.

7. Mather, LE. Dispositions of Mepivacaine and Bupivacaine Enantiomers in Sheep (Br J Anaesth. 1991; 67:239-46), vol. 1.19, p 303. Both enantiomers of both agents were cleared principally by the liver. At the doses studied (~40 mg), the enantiomers did not cause overt pharmacological effects.

8. Rutten, AJ, Mather, LE, McLean, CF, Nancarrow, C. Tissue Distribution of Bupivacaine Enantiomers in Sheep (Chirality. 1993 5:485-91), vol. 1.19, p 311. Sheep infused intravenously with fatal doses of racemic-bupivacaine had greater concentrations of (+)-(R)-bupivacaine than (-)-(S)-bupivacaine in brain (P = 0.028) and the ventricle (P = 0.036). This may interpret the greater myocardial toxicity of (R)-bupivacaine found in vitro.

9. In vitro chiral interconversion study with precision cut rat and human liver slices (CHO 1/932154), vol. 1.20, p 001. Reviewed on July 3, 1997 under IND

In human liver slices incubation, a decrease of 79% and 77% of the initial concentration in the 5 ug/ml and 10 ug/ml of levobupivacaine occurred, respectively. R(+) bupivacaine was not detected in any samples analyzed. S(-) Bupivacaine concentration in rat liver slices decreased to 63% and 83% of the initial concentrations in 5ug/ml and 10 ug/ml of bupivacaine groups, respectively. However, no R(+) bupivacaine was detected in any sample analyzed.

10. The in vitro binding of [^{14}C]-levobupivacaine and [^{14}C]-bupivacaine to the plasma proteins and the blood cells of rat, rabbit and man

Performing Laboratory:
Project No. 159721.
Sponsor: Chiroscience limited, Cambridge Science Park, Cambridge, England.
QA statement: Yes
Study Completion Date: Oct. 28, 1997
Test Compounds: [^{14}C]-levobupivacaine (Batch No. CFQ 9713, radiochemical purity 99.3%, specific activity 1.48 GBq/mmol, supplied by Amersham International Plc.).
[^{14}C]-bupivacaine (Batch No. CFQ 10009, radiochemical purity 97.7%, specific activity 1.59 GBq/mmol, supplied by Amersham International Plc.).
Phosphate buffered saline was used to prepare the stock solutions.
Animals: Male Sprague-Dawley rats, age 7-10 weeks, Supplied by
Male New Zealand white rabbits, age 3 months, supplied by
Healthy male human volunteers, age 18 - 30 years, not on medications

Results:

Binding of [^{14}C]-bupivacaine at a target concentration of 10 µg/ml with rat, rabbit and human plasma proteins.

Incubation Time (min)	Protein Bound (%)		
	Rat	Rabbit	Man
0	65.9	61.7	87.5
10	79.3	63.1	92.3
30	79.6	67.5	93.4
60	77.0	68.5	92.2
120	78.7	75.7	91.7

In vitro binding of [^{14}C]-levobupivacaine at various concentrations to rat, rabbit and human plasma proteins.

Target Concentration (µg/ml)	Protein Bound (%)		
	Rat	Rabbit	Man
0.01	84.8	73.5	97.4
0.1	85.2	69.7	98.1
1	78.6	63.6	97.9
10	61.8	58.4	84.2

In vitro binding of [^{14}C]-bupivacaine at various concentrations to rat, rabbit and human plasma proteins.

Target Concentration (µg/ml)	Protein Bound (%)		
	Rat	Rabbit	Man
0.01	85.9	68.0	100.5
0.1	83.8	72.2	98.3
1	77.7	65.0	98.3
10	58.5	62.8	90.4

The binding of [^{14}C]-levobupivacaine to human plasma proteins at a concentration of 1µg/ml was similar (>98%) in plasma at pH 7.8 and in plasma adjusted to pH 7.4

Association of [^{14}C]-bupivacaine at a target concentration of 10 µg/ml with rat, rabbit and human blood cells.

Incubation Time (min)	Associated with Blood Cells (%)		
	Rat	Rabbit	Man
0	42.6	41.5	18.3
10	43.8	41.2	33.1
30	41.0	41.1	28.3
60	45.8	40.2	27.1
120	43.9	42.3	22.5

Association of [^{14}C]-levobupivacaine at various concentrations with rat, rabbit and human blood cells.

Target Concentration (µg/ml)	% Associated with Blood Cells		
	Rat	Rabbit	Man
0.01	29.2	35.1	-1.4
0.1	31.9	26.9	0.3
1	32.5	28.1	1.9
10	65.2	30.6	32.2

Association of [^{14}C]-bupivacaine at various concentrations with rat, rabbit and human blood cells.

Target Concentration (µg/ml)	% Association with Blood Cells		
	Rat	Rabbit	Man
0.01	33.9	36.4	9.1
0.1	30.8	23.9	16.6
1	40.3	31.2	8.5
10	60.8	31.3	37.6

11. A study to investigate forms of cytochrome P450 responsible for the metabolism of [^{14}C]-levobupivacaine and [^{14}C]-bupivacaine in human liver microsomes: tentative identification using isozyme selective chemical inhibitors

Performing Laboratory:
Project No. 160501
Experimental Completion Date: Sept. 12, 1997
Sponsor: Chiroscience Limited, Cambridge Science Park, Milton Road, Cambridge, England.
Materials: [^{14}C]-levobupivacaine (Batch No. CFQ 9713, 40 mCi/mmol, 96.06 % purity), and levobupivacaine (Batch NO. 22801) both supplied by the sponsor.
[^{14}C]-bupivacaine (Batch No. CFQ9714, 45 mCi/mmol, 96.24% purity) and bupivacaine (Batch No. 32203), both supplied by the sponsor.
Human liver samples (2♂ and 2♀) -

Results:

Kinetic parameters for the formation of the major metabolite (RT ca 20 min).

Liver ID	[^{14}C]-Levobupivacaine			[^{14}C]-Bupivacaine		
	Vmax (nmol/min/mg)	Km (µM)	Vmax/Km (µl/min/mg)	Vmax (nmol/min/mg)	Km (µM)	Vmax/Km (µL/min/mg)
005	1.28	720	1.78	0.37	452	0.82
006	1.58	330	4.79	0.88	203	4.32
007	0.94	239	3.93	1.41	760	1.86
008	1.23	204	6.03	0.78	198	3.94
Pool	0.84	309	2.72	0.44	176	2.5

Inhibition of [^{14}C]-levobupivacaine (125 µM) and [^{14}C]-bupivacaine (62.5 µM) metabolism by isozyme inhibitors.

Inhibitor	Concentration (µM)	Isozyme Selectivity	% Inhibition of Metabolite formation from	
			Levobupivacaine	Bupivacaine
Furafylline	50	CYP1A2	13.83	35.01
Sulfaphenazole	100	CYP2C9	12.23	32.92
Tranylcypromine	50	CYP2C19	0	0
Quinidine	20	CYP2D6	0	0
Diethyldithiocarbamate	50	CYP2E1	0	0
Ketonazole	5	CYP3A4	76.03	78.93

12. The comparative metabolism of [^{14}C]-levobupivacaine and [^{14}C]-bupivacaine in hepatic

microsomes from rat, rabbit and man
Performing Laboratory:
Project No. 160517
Study Completion Date: Oct. 17, 1997
Sponsor: Chiroscience Limited, Cambridge Science Park, Milton Road, Cambridge, England.
Materials: [^{14}C]-levobupivacaine (Batch No. CFQ 9713, 40 mCi/mmol, 96.06 % purity), and levobupivacaine (Batch NO. 22801) both supplied by the sponsor.
[^{14}C]-bupivacaine (Batch No. CFQ10009, 43 mCi/mmol, 96.24% purity) and bupivacaine (Batch No. 32203), both supplied by the sponsor.
Human liver samples were supplied by
Sprague-Dawley Rats were supplied by
New Zealand white rabbits were obtained from

Results:

Cytochrome P450 (CYP) and alkoxyresorufin O-dealkylase (AROD) activities of microsomal suspension used in the study.

Species	Sex	CYP	EROD	PROD	MROD
Rat	M	2.00	218.01	20.98	ND
Rat	F	2.09	390.53	11.34	ND
Rabbit	F	3.32	770.22	20.68	ND
Human	M	0.92	6.59	ND	11.07
Human	F	0.44	28.42	ND	37.73

ND - Not determined
Cytochrome P450 (CYP) - nmol/mg protein
Methoxyresorufin O-deethylase (MROD) - pmol/min/mg protein
Ethoxyresorufin 0-deethylase (EROD) - pmol/min/mg protein
Pentroxyresorufin O-deethylase (PROD) - pmol/min/mg protein

The metabolite profile of both [^{14}C]-levobupivacaine and [^{14}C]-bupivacaine in male rat liver microsomes was identical with up to 5 metabolite's peaks. The overall rate of metabolism in rabbits exceeded that seen in the rat. In human liver microsomes, there was no specific metabolite to humans.

13. Metabolite profiling, enzyme deconjugation and metabolite identification studies with samples generated following administration of [^{14}C]-levobupivacaine and [^{14}C]-bupivacaine to rats and [^{14}C]-levobupivacaine to humans

Performing Laboratory:
Project No. 160873
Sponsor: Chiroscience Limited, Cambridge Science Park, Milton Road, Cambridge,

England.

Materials: [^{14}C]-levobupivacaine (Batch No. CFQ 9713, 40 mCi/mmol, 96.06 % purity), and levobupivacaine (Batch NO. 22801) both supplied by the sponsor.
[^{14}C]-bupivacaine (Batch No. CFQ9714, 45 mCi/mmol, 96.24% purity) and bupivacaine (Batch No. 32203), both supplied by the sponsor.

Urine and feces samples: Pooled urine and feces samples from Project No. 159370 "The disposition of [^{14}C]-levobupivacaine in the rat following subcutaneous administration," Project No. 159386 "The disposition of [^{14}C]-bupivacaine in the rat following subcutaneous administration," and Project No. 159585 "The excretion and plasma kinetics of [^{14}C]-levobupivacaine in man following a single intravenous administration" was used.

Results:

The metabolic profiles of [^{14}C]-bupivacaine and [^{14}C]-levobupivacaine in rats were relatively complex but identical. The metabolic profiles in urine were more complex than in feces. Only a single major metabolite was present in human feces. In contrast, up to 13 metabolites were present in human urine, more complex than in rats. Glucuronide and sulphate conjugates of hydroxylevobupivacaine were identified as major components in human urine.

3.1 Method Validation Studies

All studies were conducted at
Sponsor: Chiroscience Limited, Cambridge Science Park, Milton Road, Cambridge, England.

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information

[redacted]

IV. Carcinogenicity : None

V. Immunotoxicity: None

VI Reproductive Toxicology

1. The Placental Transfer and Effects of Levobupivacaine, Ropivacaine and Racemic Bupivacaine on the Mature Fetal Lamb

Study No: D1249-088-PH
Study Dates: March-June, 1995
Report Date: Sept. 1997.
Study Director: Alan C. Santos, Associate Professor of Anesthesiology, Obstetrics and Gynecology, Montefiore Medical Center, Bronx, New York.
Sponsor: Chiroscience Ltd.
GLP Statement: Yes
Compounds Tested: Levobupivacaine, Bupivacaine and Ropivacaine:
Doses: 0.07 mg/kg/min for 15 minutes followed by 0.035 mg/kg/min for 45 min.
Route: Intravenous
Animals: Ten ewes per group (40 - 80 kg body weight) and near the term of pregnancy (125 - 135 days), 1 - 5 years old.
Supplier:

Uterine blood flow [UBF], intra-amniotic pressure [IAP], maternal and fetal blood pressure, and heart rates were recorded during the experiment. At the end of the experiment, serum drug concentration in the mother and foetus and selected foetal tissues (cerebral cortex, cerebellum, midbrain, brainstem, myocardium, lungs, kidneys, liver, and adrenal gland) were measured.

Results:

There were no differences in UBFand IAP from base line values. There was a decrease in HR at the end of infusion in a bupivacaine group.

Maternal blood pressure and heart rates

Time (min)	MAP (mm Hg) B	L	R	CVP (mm Hg) B	L	R	HR (b/min) B	L	R
0	89.1 ±10.5	88.3 ±5.2	90.9 ±11.6	6.2 ±2.7	6.11 ±4.3	4.2 ±3.9	103 ±22.9	95 ±9.9	108 ±19.5
30	90.5 ±11.2	88.8 ±4.2	90.2 ±10.4	7.0 ±3.2	7.0 ±4.4	5.1 ±4.7	89 ±19.7	94 ±14.7	101 ±18.7
60	88.8 ±9.8	87.9 ±5.5	89.4 ±12.9	7.3 ±4.1	5.7 ±4.3	5.8 ±5.3	82 ±15.2	87 ±10.3	102 ±18.3

The difference in HR in the racemic bupivacaine was significantly different. There were no differences in a foetal blood pressure or heart rate between drug treatment groups as shown on the next page.

Time (min)	MAP (mmHg) B	L	R	HR (b/min) B	L	R
0	45.7±5.2	45.3±5.6	44.5±6.9	153±14.2	155±13.3	164±14.1
30	48.6±5.3	47.7±5.6	47.5±7.2	151±15.9	152±10.5	159±14.3
60	49.0±4.3	47.5±5.2	46.8±5.9	150±15.5	151±7.2	158±15.3

There were no significant differences among the maternal or foetal serum drug concentrations. The highest tissue levels for all three drugs were in the adrenal, liver, lung, kidney, and heart.

2. The Effects of Pregnancy on the Systemic Toxicity of Levobupivacaine, Bupivacaine and Ropivacaine in Sheep Following Repeated Cumulative Intravenous Bolus Administration

Study Dates: 15th July 1997 - 30th January 1998
Report Date: April 1998.
Study Director: Alan C. Santos, M.D., Associate Professor of Anesthesiology, Obstetrics and Gynecology, Montefiore Medical Center, Bronx, New York.
Sponsor: Chiroscience Ltd.
GLP Statement: Yes
Compounds Tested: Levobupivacaine, batch no. 0130N
Bupivacaine, batch no. 0131N and
Ropivacaine, batch no. 0132N.
Doses: An initial bolus dose of 0.67 μmol/kg followed by repeated i.v. bolus doses of 1.76 μmol/kg with 1-minute intervals between boluses until cardiovascular collapse.
Route: Intravenous bolus over a period of 60 seconds
Animals: Three groups of non-pregnant sheep (12/group, 1 - 4 years old) and three groups of pregnant sheep (12/group, 120 - 142 gestation days)
Supplier:

Results: There were no statistical differences in the accumulated dose or accumulated time to onset of toxic events between pregnant and non-pregnant sheep for all three local anesthetics tested.

Mean or Median Data at Cardiovascular Collapse

Animal/Drug	Mean Accumulated Dose (mmol/kg) at Cardiovascular Collapse	Mean Time to Onset of Cardiovascular Collapse (min)	Median Total Serum Concentration of Drug (µg/ml) at Cardiovascular Collapse	Median Serum Concentration of Free Drug (µg/ml) at Cardiovascular Collapse	Median Percentage Protein Binding (%) at Cardiovascular Collapse
Non Pregnant					
Levobupivacaine	0.044±0.021	50.25±23.68	5.149	1.938	52.31
Ropivacaine	0.0640.025	77.10±28.81	5.505	3.152	32.14
Bupivacaine	0.029±0.014	33.74±15.50	2.320	1.006	53.82
Pregnant					
Levobupivacaine	0.035±0.014	39.20±17.47	5.157	2.845	47.00
Ropivacaine	0.058±0.028	66.73±31.62	6.672	4.903	31.70
Bupivacaine	0.033±0.012	38.55±13.73	2.537	0.889	49.34

Overall, mean dose and time to the event were lowest for bupivacaine and highest for ropivacaine.

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Accumulated dose (AD, mmol/kg) and Accumulated time to onset (TTO, min) data for each toxic event [Mean ± SD]

	Convulsion	Hypotension	Circulatory Collapse
Non-pregnant			
Levobupivacaine AD(mmol/kg) TTO (min)	0.028±0.017 32.66±19.86	0.05±0.02 57.80±22.3	0.044±0.02 50.25±23.68
Ropivacaine AD(mmol/kg) TTO (min)	0.032±0.005 37.16±6.16	0.058±0.019 66.78±21.32	0.064±0.025 77.10±28.81
Bupivacaine AD(mmol/kg) TTO (min)	0.018±0.008 21.59±9.08	0.033±0.011 38.31±12.90	0.029±0.014 33.74±15.50
Pregnant			
Levobupivacaine AD(mmol/kg) TTO (min)	0.021±0.007 22.88±6.67	0.041±0.017 47.42±19.74	0.035±0.014 39.20±17.47
Ropivacaine AD(mmol/kg) TTO (min)	0.032±0.021 36.79±23.51	0.060±0.027 69.52±30.84	0.058±0.028 66.73±31.62
Bupivacaine AD(mmol/kg) TTO (min)	0.017±0.005 20.04±5.78	0.038±0.011 43.99±12.25	0.033±0.012 38.55±13.73

Animals receiving bupivacaine reached convulsion and hypotension more quickly and with lower doses than those receiving levobupivacaine. Animals receiving ropivacaine reached circulatory collapse at higher doses than the other two treatments.

There was no difference in heart rate, mean arterial pressure, arterial blood pH or arterial blood PCO_2 and PO_2 between non-pregnant and pregnant animals. The pregnancy did not increase the sensitivity to the toxicity of the drugs (bupivacaine, levobupivacaine and ropivacaine).

3. Levobupivacaine: subcutaneous preliminary study in the non-pregnant rat

Test Facility:(
Report Number: 1193/84-1050
Report Issue: October 1996
GLP/QA Statements: Yes
Animals: Crl:CDBR rats, 10 to 12 weeks old, 224 to 265 g body weight.

Supplier:
Test Article: Levobupivacaine, batch No. 22801 (100% pure)
Control Article: 0.9% saline
Route: Subcutaneous
Dosing: Groups 2 to 4 - 14 days, Control - 21 days

Group Number	Group Description	Dose level (mg/kg/day)	Dose conc. (mg/ml)	Dose volume (ml/kg)	Number of females	Duration of treatment (Days)
1	Control	0	0	5	3	21
2	Low	10	2	5	3	14
3	Intermediate	20	4	5	3	14
4	High	30	6	5	6	14
5	High 2	40	8	5	3	2

Treatment for group 5 animals was ended after 2 days because one animal was killed in extremis and the other had repeated convulsive episodes.

Results:

Morbidity: Groups 1 to 4 - None
Clinical Signs: Group 4 - One animal had discolored eyes and tears from the eye (ocular swelling)
Group 5 - Impaired mobility
Body Weight, Food Intake: No effect
Necropsy: Reddening at the injection sites in all treated animals.

A dosage of 30 mg/kg/day was recommended for use as the high dose in the reproductive toxicity studies.

4. Levobupivacaine and bupivacaine: subcutaneous study of fertility and embryo-fetal development in the rat

Test Facilities:

Report No.: CLE 1193/86 - 1050
Report Issue: Sept. 29, 1997
GLP/QA Statements: ICH Harmonised Tripartite Guidelines
Animals: Crl:CDBR strain rats
Testing animals: 24/group/sex.
Satellite animals: 9/group/sex for toxicokinetics
Males: 10 - 12 weeks old, 371 - 471 g body weight
Females: 12 - 14 weeks old, 239 - 325 g body weight.
Supplier:

Test and control articles: Levobupivacaine, batch number 22801
Bupivacaine, batch number 32203
0.9% saline
Route: Subcutaneous
Dosing: Males - Four weeks before mating
Females - Two weeks before mating, during pairing and until day 17 of gestation.

Group Number	Test Article	Group Description	Dose Level (mg/kg/day)	Dose Concentration (mg/ml)	Dose Volume (ml/kg)
1	-	Control	0	0	5
2	Levobupivacaine	Low	10	2	5
3	Levobupivacaine	Intermediate	20	4	5
4	Levobupivacaine	High	30	6	5
5	Bupivacaine	Comparator	10	2	5

Necropsy: Males - Week 12
Females - Day 20 of gestation
Satellite groups - Day 15 of the pre-pairing treatment period.
Parameters: Standard

Results:

Morbidity and mortality: Group 2 - 1♂ and 1♀
Group 4 - 2♂, 3♀, Group 5 - 1♂
Clinical observation: Dose-related increase in sores/lesions at the injection sites, rough hair-coat and/or hair loss both in males and females.
Body weight: No effect
Food intake: No difference
Oestrus and mating data: No drug or dose-related differences.
Uterine/implantation data: ↓Corpora lutea in group 4 (high dose), no differences in pre- and post-implantation loss.
Foetal Data: Mean foetal, litter and placental weights - No differences
External, visceral and skeletal malformations - No dose-related differences
Seminology data: No differences in sperm motility, count, proportion of morphological abnormalities or on the concentration. ↓ Mean sperm concentration in group 5 (bupivacaine).
Necropsy and histopathology:
Macroscopic findings: Dose-related increases in redness and/or sores, showing some degree of irritation at the injection site.
Microscopic findings: No evidence of test article toxicity
Testicular staging: No difference

Toxicokinetic: Vol. 1.19, p 1 - 72

Sponsor: Chiroscience limited, Milton Road, Cambridge, England.
Performing Laboratory: [redacted]
Project No.: 363925
Quality Assurance Statement: Yes
Samples taken: On days 1 and 14 of the study.

Dosing with 10 mg/kg/day levobupivacaine

Parameter	Day 1		Day 14	
	Males	Females	Males	Females
C_{max} (µg/ml)	0.545	0.242	0.398	0.482
T_{max} (h)	0.5	2.0	1.0	0.25
$T_{1/2}$ el* (h)	1.27	-	-	-
AUC $_{0-t}$ (h.µg/ml)	0.5491	0.3841	0.5990	0.6170
AUC $_{0-\infty}$* (h.µg/ml)	0.9496	-	-	-

- = Not calculated
* = These parameters estimates should be considered unreliable guesses and are reported here for guidance.

There was no evidence of accumulation of either R- or S-bupivacaine with daily dosing of bupivacaine for more than 14 days.

Dosing with 20 mg/kg/day levobupivacaine

Parameter	Day 1		Day 14	
	Males	Females	Males	Females
C_{max} (µg/ml)	1.114	0.737	0.934	1.023
T_{max} (h)	0.50	0.25	0.50	0.25
$T_{1/2}$* el (h)	2.86	3.55	2.63	1.62
AUC $_{0-t}$* (h.µg/ml)	1.51	0.9908	1.2050	1.2718
AUC $_{0-\infty}$* (h.µg/ml)	4.5516	3.441	3.2583	2.2125

Dosing with 30 mg/kg/day levobupivacaine

Parameter	Day 1 Males	Day 1 Females	Day 14 Males	Day 14 Females
C_{max} (µg/ml)	1.472	1.023	1.205	1.348
T_{max} (h)	0.25	0.50	0.50	2.0
$T_{1/2}$* el (h)	-	1.70	1.87	-
AUC_{0-t} (h.µg/ml)	2.0353	1.5486	1.9814	1.7740
$AUC_{0-\infty}$* (h.µg/ml)	-	2.9146	3.9149	-

The parameter estimates for R- and S-bupivacaine after dosing with 10 mg/kg/day bupivacaine

Parameter		Day 1 R-bupivacaine	Day 1 S-bupivacaine	Day 14 R-bupivacaine	Day 14 S-bupivacaine
C_{max} (µg/ml)	Males	0.229	0.507	0.221	0.319
	Females	0.171	0.209	0.140	0.189
T_{max} (h)	Males	1.0	0.5	1.0	1.0
	Females	0.25	0.25	1.0	1.0
$T_{1/2}$ el * (h)	Males	-	-	65.22	-
	Females	1.297	0.933	2.23	1.69
AUC_{0-t} (h.µg/ml)	Males	0.350	0.509	0.342	0.454
	Females	0.235	0.107	0.227	0.299
$AUC_{0-\infty}$*(h.µg/ml)	Males	-	-	17.18	-
	Females	0.3623	0.244	0.510	0.554

Levobupivacaine dosing produced twice the systemic exposure (C_{max} and AUC_{0-t}) to S-bupivacaine than does dosing with racemic bupivacaine. There was no evidence of accumulation of either R- or S-bupivacaine with daily dosing of bupivacaine over 14 days.

5. Bupivacaine HCl & S-Bupivacaine HCl: Study to Compare Effects on Embryo-Fetal Development in the Rat Reviewed on July 3, 1997 under IND

Treatment of Crl:CD^R rats from Day 6 to Day 15 of pregnancy with bupivacaine or levobupivacaine at 6 or 18 mg/kg/day subcutaneously did not produce any drug-related effects on the pregnant females or developing litters. The malformation (~9/300) showed squat foetus (6), diaphragmatic hernia, micro/anophthalmia with retinal folding or small orbital socket. There were no obvious differences between the racemate and the S-enantiomer.

6. Levobupivacaine and bupivacaine: subcutaneous study of pre- and postnatal development in the rat

Report for: Chiroscience Limited, Cambridge, UK.
Test Facilities:

Report No.: CLE 1193/85 - 1050
Report Issue: Sept. 29, 1997
GLP/QA Statements: Yes
Animals: Mated Crl:CDBR strain rats
Testing animals: 24/group.
Satellite animals: 9/group for toxicokinetics
Supplier:
P1 females were 10-12 weeks old at the time of mating.
F1 generation animals were 40 to 49 days old at the start of the maturation period.
Test and control articles: Levobupivacaine, batch number 22801
Bupivacaine, batch number 32203
0.9% saline
Route: Subcutaneous
Dosing: P females - From Day 6 of gestation to Day 21 postpartum.

Group Number	Test Article	Group Description	Dose Level (mg/kg/day)	Dose Concentration (mg/ml)	Dose Volume (ml/kg)
1	-	Control	0	0	5
2	Levobupivacaine	Low	10	2	5
3	Levobupivacaine	Intermediate	20	4	5
4	Levobupivacaine	High	30	6	5
5	Bupivacaine	Comparator	10	2	5

Necropsy: F1 males - in week 16
Mated F1 females - Day 13 of gestation
Satellite groups - After final blood samples on day 11 or 12 of gestation.
In addition to standard parameters, the following development parameters were measured for each letter: Pinna unfolding, incisor eruption, eye opening, surface righting reflex , air righting reflex, grip strength, pupillary reflex, auditory response, and visual placing response. F1 learning ability, F1 motor activity, F1 generating mating and F1 caesarian data were also

collected.

Results:

Morbidity and mortality: Group 2 - one animal died
Group 4 - 3 females died (on day 1 and 9 of lactation and on day 6 of gestation)
Clinical observation: Dose-related increase in sores/lesions at the injection sites, rough hair coat and/or hair loss.
Body weight: No effect
Food intake: No difference
Gestation, post-implantation survival, live birth and viability indices and physical and functional development of the offspring to weaning were similar to the control group.

F1 generation - Clinical observation, body weight, food intake, physical development and mating performance - no differences

Toxicokinetics: Vol. 1.18, p 239 - 277

Performing Laboratory:
Project No.: 363925
Report No.: 15149A
Quality Assurance Statement: Yes

Parameter	Dose			
	10 mg/kg/day levobupivacaine	20 mg/kg/day levobupivacaine	30 mg/kg/day levobupivacaine	10 mg/kg/day bupivacaine
C_{max} (μg/ml)	1.453	1.128	1.953	0.867
T_{max} (h)	0.50	0.25	0.25	0.50
$T_{1/2}el$ (h)*	1.20	-	1.90	0.72
AUC_{0-t} (h.μg/ml)	1.118	1.195	1.933	0.505
$AUC_{0-\infty}$ (h.μg/ml)*	1.724	-	4.099	0.654

- = Not calculated
* = These are unreliable estimates and are reported here for guidance only.

No accumulation of either R- or S-bupivacaine with daily dosing over 11 days.

7. Levobupivacaine: subcutaneous preliminary study in the non-pregnant rabbit

Report Number: 1193/99-1050

Prepared for: Chiroscience Limited, Cambridge, CB4 4WE, England.
Issue Date: Dec. 10, 1996
Test Facility:
Test Compound: Levobupivacaine, batch No. 22801.
GLP/QA Statements: Yes

Group Number	Group Description	Dose Level (mg/kg/day)	Dose Concentration (mg/ml)	Dose Volume (ml/kg)	Number of Females
1	Control	0	0	5	3
2	Low	20	4	5	3
3	High	30	6	5	3

Two of the three animals in 30 mg/kg group had convulsive episodes with associated effects; one animal died after eight minutes. Third animal was subdued with impaired mobility. The treatment of group 3 was stopped after the initial dose. Treatment of control and group 2 continued for 14 days. Based on the resuls of the study, it was suggested that dose levels of 5, 10 and 20 mg/kg/day levobupivacaine be used for final study.

8. Levobupivacaine and bupivacaine: subcutaneous study of embryo-fetal development in the rabbit

Report for: Chiroscience Limited, Cambridge, UK.
Test Facilities:

Report No.: CLE 1193/100-1050
Report Issue: Sept. 29, 1997
GLP/QA Statements: Yes (ICH Harmonised Tripartite Guidelines, 24 June 1993)
Animals: New Zealand White rabbits (Crl.NZW/Kbl BR strain)
Animals were 4 to 7 months old, at least 2.5 kg body weight.
Supplier:
Testing animals: 24/group.
Satellite animals: 3/group for toxicokinetics
Test and control articles: Levobupivacaine, batch number 22801
Bupivacaine, batch number 32203; 0.9% saline
Route: Subcutaneous
Dosing: From Days 7 to 19 of gestation.

Group Number	Test Article	Group Description	Dose Level (mg/kg/day)	Dose Concentration (mg/ml)	Dose Volume (ml/kg)
1	-	Control	0	0	5
2	Levobupivacaine	Low	5	1	5
3	Levobupivacaine	Intermediate	10	2	5
4	Levobupivacaine	High	20	4	5
5	Bupivacaine	Comparator	5	1	5

Blood samples: From nine of the main study and three satellite animals per group on Day 12 of gestation.
Necropsy: Main study animals - Day 29 of gestation
Satellite groups - After final blood samples on day 13 of gestation.

Results:

Morbidity and mortality: Group 2 - one animal was killed on Day 20 of gestation.
Group 4 - 3 females killed (on Days 12, 14 and 19 of gestation).
Group 5 - one animal was killed (Day 19 of gestation).
Clinical observation: High dose animals - Convulsive episodes followed by rapid respiration, splayed gait and subdued behavior during days 11 to 19 of gestation.
Body weight: High dose animals - ↓1.6%
Food intake: High dose animals - Lower
Uterine/implantation data: High dose group - Higher incidences of pre-implantation loss
Foetal Data: The proportion of male foetuses in the high dose levobupivacaine (44%) and Comparator bupivacaine (44%) group was lower than the concurrent control (53%).
Foetal defect data: No dose-related effects on external, visceral and skeletal malformations.

Toxicokinetics: Vol. 19, p 073.

Performing Laboratory:
Project No.: 364164
Sponsor: Chiroscience Limited, Cambridge, England.
Quality Assurance Statement: Yes
The plasma samples were taken on Day 12 of gestation.

Parameter	Dose				
	5 mg/kg Levobupivacaine	10 mg/kg Levobupivacaine	20 mg/kg Levobupivacaine	5 mg/kg Bupivacaine	
				R-Bupivacaine	S-Bupivacaine
T_{max} (h)	2.0	0.50	0.50	0.50	0.50
C_{max} (µg/ml)	0.346	0.572	1.123	0.346	0.142
$T_{1/2}$ el* (h)	-	1.47	5.18	-	5.73
AUC_{0-t} (h.µg/ml)	0.5974	0.8398	1.5254	0.5974	0.2368
$AUC_{0-\infty}$ (h.µg/ml)	-	1.4403	8.1270	-	1.292

- = Not calculated
* = These parameters should be considered unreliable estimates and are reported here for guidance only.

9. Toxicokinetic Analysis of Rat Plasma Sample from Pre/Post Natal Reproductive Toxicity
Please see under original study on page 42.

10. Toxicokinetic Analysis of Rat Plasma Samples from a Fertility and Embryofetal Study
See under original study on page 38.

11. Toxicokinetic analysis of plasma samples from a rabbit embryofetal toxicology study
See under original study on page 44.

12. [^{14}C]-Levobupivacaine: Comparative foetal tissue distribution with [^{14}C]-bupivacaine using quantitative whole body autoradiography following subcutaneous administration (1 mg/kg) to the pregnant rat and rabbit and the post partum rat

Sponsor: Chiroscience Limited, Cambridge Business Park, Cambridge, England.
Test Facility:

CHE Report no: 1193/125-1006
Date of issue: 16 September 1997
GLP/QA statements: Yes
Test articles: ^{14}C-Levobupivacaine (batch no. CFQ9713, Amersham International).
^{14}C-bupivacaine (batch no. CFQ9714, Amersham International).
Levobupivacaine (batch no. 22801, sponsor) and

Bupivacaine (batch no. 32203, sponsor).
Species: Sprague-Dawley rats (time-mated, 9 -10 weeks old)
Crl.NZW/Kbl rabbits (time-mated, 5 - 6 months old)

Dose group	Animal No./ species	Test article	Animal status	Dose administered mg/kg	µCi/kg	MBq/kg
A	5/Rat	^{14}C- levobupivacaine	Day 18 of gestation	1.00 - 1.01	86.2 - 87.0	3.19 - 3.22
B	3/Rat	^{14}C- levobupivacaine	Day 2 post-partum	0.99 - 1.01	85.1 - 86.6	3.15 - 3.20
C	5/Rat	^{14}C- bupivacaine	Day 18 of gestation	0.99 - 1.02	78.7 - 81.2	2.91 - 3.01
D	3/Rat	^{14}C- bupivacaine	Day 2 post-partum	1.00	84.4 - 84.5	3.12 - 3.13
E	5/Rabbit	^{14}C- levobupivacaine	Day 20 of gestation	0.98 - 1.01	84.5 - 86.6	3.12 - 3.20
F	4/Rabbit	^{14}C- bupivacaine	Day 20 of gestation	1.00 - 1.01	78.9 - 79.5	2.92 - 2.94

Results:

Radiochemical purity: [^{14}C]-Levobupivacaine - 96.9%
[^{14}C]-bupivacaine - 99.1%
Specific activity: [^{14}C]-levobupivacaine - 118.7 µCi/mg (4.39 MBq/mg)
[^{14}C] -bupivacaine - 119.4 µCi/mg (4.42 MBQ/mg)
Tissue distribution of activity:

Group A - Peak concentrations occurred in most tissues - 2 hour post-dose.
Peak concentration in foetal tissues - 0.5 hour post-dose.
Blood concentrations were 70.4, 90.8 and 46.3 ng equiv./g at 0.5, 2.0 and 6.0 h post-dose. Maximum concentrations in the liver and kidney occurred at 2 h (668.3, 520.0 ng.equiv/g, respectively).

Group B - Tissue concentrations in the neonates were below measurable radioactivity (limit of quantification = 26.7 ng equivalents of levobupivacaine/g of tissue).
Group C - Similar to group A except adrenal, thyroid and clitoris.

Tissue	Ng of test article/g of tissue kill time 0.5 h		2 h		6 h		24 h	
	DGA	DGC	DGA	DGC	DGA	DGC	DGA	DGC
Adrenal cortex	1365	909	1785	1098	1371	1162	567	283
Adrenal medulla	908	414	1419	528	988	542	74	165
Thyroid	608	472	732	505	451	371	177	106
Clitoris	599	NS	1224	841	1058	597	254	79

DGA - animals administered with ^{14}C-levobupivacaine
DGC - animals administered with ^{14}C-bupivacaine

Blood concentrations in the dams were as follows.

Test article	Ng equivalent of test article/g of blood 0.5 h	2 h	6 h	24 h
^{14}C-levobupivacaine	70.4	90.8	46.3	Below the limit
^{14}C-bupivacaine	63.4	101.9	116.2	38.4

Group D - Similar to group B, radioactivity concentrations in all neonate tissues were below the quantification.
Group E - Mean concentrations of radioactivity in the tissues of rabbit foetuses

Tissues	Mean ng equivalents of levobupivacaine/g of tissue Kill time 0.5 h	2 h	6 h	24 h
Blood	NQ	64.2	67.3	NQ
Brain	40.0	80.8	77.5	NQ
Eye	14.9	57.3	72.5	NQ
kidney	15.7	77.0	109.9	41.2
Liver	70.8	139.4	134.3	NQ
Lung	28.0	69.0	79.3	NQ
Myocardium	7.9	70.7	78.9	NQ
Skin	5.8	46.3	59.1	NQ
Placenta	97.3	158.1	191.3	67.8

NQ - Below the limit of quantification

All foetal blood concentrations were below the limit of quantification

Group F -

Mean concentrations of radioactivity in the tissues of rabbit foetuses

Tissues	Mean ng equivalents of bupivacaine/g of tissue Kill time 0.5 h	2 h	6 h	24 h
Blood	NA	135.8	71.1	NQ
Brain	NA	186.7	82.5	NQ
Eye	NA	128.6	68.9	NQ
kidney	NA	201.7	113.7	6.0
Liver	NA	272.9	133.5	NQ
Lung	NA	162.5	73.7	NQ
Myocardium	NA	172.4	74.3	NQ
Skin	NA	184.3	56.0	NQ
Placenta	59.6	441.8	207.3	NQ

NQ - Below the limit of quantification NA - Not applicable

VII.Genotoxicology

1. S Enantiomer Bupivacaine HCL Bacterial Mutation Assay
Reviewed on July 3, 1997 under IND

S-enantiomer bupivacaine at 5000 ug/plate was not toxic toward the tester strains. No substantial increases in a revertant colony were observed in the presence or absence of S-9 mix, in either mutation test. The positive control showed the sensitivity of the test.

2. In Vitro Levobupivacaine HCL: Mutation at the thymidine kinase (tk) locus of mouse lymphoma L5187Y cells using the Microtitre fluctuation technique

Report number: 1193/35-1052
Issue date: February 1997
Report for: Chiroscience Ltd., Cambridge, CB4 4WE, England
Test facility:

Negative control: Saline for injection
Positive control: Benzo(a)pyrene and 4-nitroquinoline 1-oxide
GLP/QA statements: Yes
After cytotoxicity range-finder experiments, the ability of levobupivacaine and bupivacaine to induce mutation at the tk locus (5-trifluorothymidine resistance) was determined in two independent experiments.

Results

Experiment 1 Levobupivacaine

Treatment (μg/ml)	-S-9 %RS	-S-9 Mutant frequency#	Treatment (μg/ml)	+S-9 %RS	+S-9 Mutant frequency#
Zero	100	118.8	zero	100	114
31.25	101.5	116.3	31.25	91.1	78.6
62.5	98.5	115.3	62.5	89.8	80.3
125	103.1	95.4	125	85.8	77.7
250	91.5	100.2	250	77.5	129.1
500	80.9	147.4	500	41.3	158.2
1000	88.2	-	1000	1.0	-

Experiment 2 Levobupivacaine

Treatment (μg/ml)	-S-9 %RS	-S-9 Mutant frequency#	Treatment (μg/ml)	+S-9 %RS	+S-9 Mutant frequency#
0	100	61.87	0	100	83.1
62.5	92.9	53.5	62.5	76.3	57.2
125	86.4	37.2	125	78.5	84.9
250	82.2	64.9	250	67.4	46.2
500	85.8	69.0	500	36.0	54
750	85.8	-	750	42.3	75.6
1000	90.2	-	1000	44.6	67.4

Experiment 1 Bupivacaine

Treatment (μg/ml)	-S-9 %RS	Mutant frequency#	Treatment (μg/ml)	+S-9 %RS	Mutant frequency#
0	100	118.8	0	100	114.3
250	103.1	104.7	250	54.3	109.2
500	92.2	106.0	500	16.5	-
750	75.4	97.1	-	-	-

Experiment 2 Bupivacaine

Treatment (μg/ml)	-S-9 %RS	Mutant frequency#	Treatment (μg/ml)	+S-9 %RS	Mutant frequency#
0	100	61.9	0	100	83.1
500	82.8	80.2	250	84.6	64.2
750	46.0	130.5*	500	50.8	88.93

- Per 10^6 viable cells * - significant at 5% level
Mutant frequency = [PE (mutant)/PE (viable)] x 10^6

3. Levobupivacaine: Induction of Chromosome Aberrations in Cultured Human Peripheral Blood Lymphocytes Reviewed on July 3, 1997 under IND

In two experiments performed, the frequencies of cells with structural chromosomes aberrations in the levobupivacaine treated cells were in the normal range and similar to concurrent solvent control cultures. The numerical aberrations observed in levobupivacaine treated cultures were statistically significantly different from frequencies observed in concurrent control levels. The biological importance of this observation is not clear.

4. In Vivo Levobupivacaine HCL: Induction of micronuclei in the bone marrow of treated mice

Report number: 1193/37-1052
Issue date: April 1997
Report for: Chiroscience Ltd., Cambridge, CB4 4WE, England
Test facility:
GLP/QA statements: Yes

After initial toxicity range-finding study, levobupivacaine was administered subcutaneously at

11.25, 22.5 or 45 mg/kg/day to groups of five male and five female mice for two consecutive days. Bupivacaine was administered at 45 mg/kg/day by the same route and duration. The animals were killed 24 or 48 hours after the second administration. Cyclophosphamide was used as a positive control.

Mice treated with levobupivacaine or bupivacaine exhibited a group ratio of PCE to NCE and frequencies of micronucleated PCE similar to vehicle control animals.

VIII. Special Toxicology Studies: See the list of Ongoing Nonclinical Studies on page 3.

Summary and Evaluation of Pharmacology Studies

Bupivacaine is an amino amide local anesthetic first synthesized by Ekstam in 1957. It is a lipid soluble and protein bound compound. Bupivacaine hydrochloride, a 50:50 racemic mixture of levobupivacaine (+S) and dexbupivacaine (-R), has been marketed as a local anesthetic since 1970. Levobupivacaine is not marketed in any country.

Efficacy: Dyhre et al (Acta Anesthesiol Scand;41:1346-1352) in 1997 reported that ropivacaine, bupivacaine, levobupivacaine, and pethidine at equimolar doses produced similar duration of sensory blockades in a peripheral nerve block in Sprague-Dawley rats. It has also been shown that all three types of bupivacaine, S(-)-bupivacaine, R(+)-bupivacaine and racemic bupivacaine produced similar inhibition of sciatic nerve-mediated functions (motor, proprioceptive and nociceptive) of the rat in vivo. Full block of all functions was achieved by all drugs at the highest dose tested (0.1 ml of 0.5%).

Safety Pharmacology: Cardiovascular Effects

1. In Vitro data

S(-)-bupivacaine affects V_{max} and action potential duration (APD) in the guinea-pig papillary muscle less than the R(+)-enantiomer at different rates of stimulation and resting membrane potentials [Vanhoutte et al., Br. J. Pharmacol (1991), 103, 1275-1281]. Guinea-pig (ventricular) myocardium showed a similar sensitivity to human (atrial) myocardium in negative inotropic effect produced by levobupivacaine, bupivacaine or ropivacaine. In guinea-pig myocytes, bupivacaine showed a significant negative inotropic effect at a lower concentration than did levobupivacaine or ropivacaine. The recovery of contractile force on the washout of local anesthetic from cardiac myocytes was 24% greater for levobupivacaine than bupivacaine.

The developed force of papillary muscles isolated from guinea-pig hearts decreased by 25% at 3 μM and 70% at 30 μM for levobupivacaine, bupivacaine and ropivacaine anesthetics. At 3 μM, only bupivacaine affected cardiac action potential, decreasing V_{max} significantly by 11%. The bupivacaine-induced block of the inactivated state of the sodium channels in guinea pigs ventricular myocytes displayed stereoselectivity. The R(+)-bupivacaine interacted faster and

more potently than S(-)-bupivacaine. Both enantiomers also bind with high affinity to the activated or open state of the channel. This interaction did not display stereoselectivity. The higher potency of R(+)-bupivacaine to block the inactivated state of cardiac Na^+ channel may explain its higher toxicity than S(-)-enantiomer (Circulation 1995; 92:3014-3024).

Bupivacaine produced an enantiomer-specific effect to delay atrioventricular conduction in the isolated perfused guinea pig heart. The (+)-bupivacaine significantly prolonged AV conduction compared with the racemate or (-)-isomer at all concentrations tested (Anesthesiology 1997;86:410-419). The QRS widening and the occurrence of severe arrhythmias was less pronounced in the isolated rabbit hearts receiving S(-) isomer than the hearts receiving the R(+) isomer or the racemic mixture.

2. In vivo data:

Rapid intravenous administration of d(+)-bupivacaine (2 mg/kg) in adult Sprague-Dawley rats produced bradycardia, hypotension, and ventricular arrhythmias. In contrast, 2 mg/kg of l(-)-enantiomer of bupivacaine showed weaker cardiotoxicity (Regional Anesthesia 1992;17:311-316).

Subconvulsive doses of bupivacaine and levobupivacaine produced similar time- and dose-dependent depression of left ventricular dP/dt_{max} (mean maxima ~25% at 37.5 mg) in adults ewes. Convulsive doses reflected the state of CNS excitation. A difference of around 25 mg in greater tolerated doses of levobupivacaine for similar CNS excitation was found. Both drugs at doses <75 mg did not produce electrocardiological effect. Bupivacaine at >75 mg or levobupivacaine at >100 mg induced ventricular arrhythmias. Three animals out of seven died within 10 minutes of receiving 150, 150, and 200 mg bupivacaine, respectively, from the sudden onset of ventricular tachycardia (VF) leading to ventricular fibrillation (VF). Similar findings have been reported from studies in dogs, pigs and rats. R(+)-bupivacaine has higher mean total body clearance and shorter half-life than S(-)-bupivacaine.

Intravenous infusion of levobupivacaine in conscious adult female sheep produced mortalities. Fatal ventricular fibrillation was found to occur at doses of 300 mg (two animals) and 350 mg (one animal) out of eight animals. Ventricular tachycardia and associated failure to fill was the cause of death in three animals at doses 250 mg (two animals) and 300 mg (one animal). Electro-mechanical dissociation was the cause of death in one animal at 350 mg and another animal at 250 mg. The mean terminal half life of levobupivacaine was 70±29 minutes.

The electrophysiological effects of Intracoronary racemic bupivacaine, levobupivacaine and ropivacaine were investigated in pentobarbital-anesthetized, ventilated swine by Reiz et al. in Switzerland (Study No. D1249-056-PH, Vol. 12, p 074) in 1997. The local anesthetics were delivered directly to the myocardium via a catheter in the left anterior descending coronary artery. This study excluded the possibility of centrally mediated electrophysiological effects. The lethal dose for bupivacaine (5.0±0.8 mg) was lower than both levobupivacaine (7.9±0.9) and ropivacaine (8.2±2.4 mg). QRS prolongation occurred with 3, 4 and 5 mg of bupivacaine, levobupivacaine and ropivacaine, respectively. Levobupivacaine produced the greatest QRS prolongation. Considering comparable anesthetic potencies of bupivacaine and levobupivacaine, the latter showed less cardiotoxicity in this study.

Summary and Evaluation of Toxicology Studies

The estimated median intravenous lethal doses (LD_{50}) for male rats were 4.35 and 4.97 mg/kg for bupivacaine and S-bupivacaine, respectively. During this acute intravenous toxicity study, the deaths occurred within half an hour of the dosing.

The local tolerance and neurotoxic potential of single epidural injection of the S-bupivacaine (levobupivacaine) in beagle dogs was compared with racemic mixture (bupivacaine). In this study, the epidural administration of a single dose of 0.2 ml/kg of 0.75% of either drug solution did not show any neurotoxicity. Treatment of female beagle dogs with epidural (racemate or S-enantiomer) bupivacaine injection of 0.75% at 0.2 ml/kg did not show any neurotoxicity. This dose did induce clinical anesthesia of acceptable duration.

There was no evidence of neurotoxicity associated with three doses (one week interval between doses) of epidural injections at 0.2 ml/kg of a 0.75% solution of levobupivacaine or bupivacaine. However, the duration of anesthetic effect may be clearly related to the total volume injected based on body weight.

Cynomolgus monkeys treated with single intrathecal administration of 1.5 or 3.0 mg/animal of levobupivacaine did not reveal any clinical toxic effects

Summary and Evaluation of ADME Studies

Absorption: The studies conducted by the sponsor and literature reports show that both bupivacaine and levobupivacaine are rapidly absorbed and widely distributed in rats, rabbits, sheep, and humans.

The in vitro plasma protein binding increased with decreasing concentrations of [^{14}C]-levobupivacaine or bupivacaine in rats, rabbits and humans. The highest protein binding was observed in human with 97% and 100% bound at low concentration (0.01 µg/ml) of levobupivacaine and bupivacaine, respectively. The plasma protein binding was similar (>98%) in human plasma samples analyzed fresh (without freezing) or following storage at -20°C for 24 h.

Distribution: Volume of distribution (24,834±3910 ml/kg for bupivacaine and 7559±3365 ml/kg for levobupivacaine) and clearance values (bupivacaine 6.5±0.2 ml/min; levobupivacaine 3.6±0.6 ml/minute) were very similar suggesting that both compounds were widely distributed in rats.

The tissue distribution of radioactivity of single doses [^{14}C]-levobupivacaine and [^{14}C]-bupivacaine in the pregnant albino rat, pregnant rabbit and post-partum rat was similar. Radioactivity was shown to be present in the tissues of suckling rats, showing that radiolabelled material partitioned into milk of the dam and was subsequently ingested by the neonate and absorbed from the gastrointestinal tract.

Metabolism:

Incubation of rat and human liver slices in the presence of S(-) bupivacaine did not provide any evidence of the chiral conversion of S(-) bupivacaine to R(+) bupivacaine. The metabolic profile of [^{14}C]-bupivacaine and [^{14}C]-levobupivacaine was also essentially identical in rats showing no enantiomeric specific metabolism.

The urinary radioactivity profiles in F344 rats showed 17 radioactive peaks following ^{14}C-bupivacaine administration and 15 radioactive peaks after ^{14}C-levobupivacaine administration. Most of the radioactivity in the feces was found in the M16 peak of both compounds.

The metabolic fate of both [^{14}C]-levobupivacaine and [^{14}C]-bupivacaine in human liver microsomes seemed identical. Ketoconazole was a potent metabolic inhibitor (~77%) showing the involvement of CYP3A4 in the metabolism of both compounds. The CYP1A2 and CYP2C9 were also involved in the metabolism. The in vitro metabolism of both [^{14}C]-levobupivacaine and [^{14}C]-bupivacaine in human liver microsomes is less complex than in microsomes from either rabbit or rat, and may be no metabolite that is specific to humans.

In human, the urinary metabolite profile of [^{14}C]-levobupivacaine was more complex than observed for rats. Up to 13 metabolites can be seen. The major route of the metabolism in humans might be hydroxylation and conjugation to either sulphate or glucuronate.

Elimination: Bupivacaine and levobupivacaine following intravenous administration in rats were rapidly excreted (bupivacaine $t_{1/2}\beta$ 11.1±1.8 h; levobupivacaine $t_{1/2}$ β 6.3. ±2.0 h). The clearance values were 6.5±0.2 ml/min for bupivacaine and 3.6±0.6 ml/min for levobupivacaine. Approximately half the radioactivity was voided in the urine and the remaining half in the feces over the five days of the study. Most of 3-hydroxylevobupivacaine, which was the major metabolite (approximately 26%), was excreted in the conjugated form.

The excretion of bupivacaine and levobupivacaine following a single subcutaneous administration were generally similar in male and female Sprague Dawley rats. The major route of excretion (~60%) was via feces suggesting the excretion of absorbed radiolabelled components via bile. A further (~30%) was excreted in urine. Overall recoveries of total radioactivity were 94 - 99% over the collection period.

The major route of excretion of [^{14}C]-levobupivacaine following subcutaneous administration in rabbits was via urine accounting for ~74% of total radioactivity. Overall recoveries of radioactivity within 24 hours after administration were 91 - 96% of the administered dose. Maximum peak concentration in the plasma was 1.1 μg equiv./ml at 1 hour after dosing and no radioactivity remained in the body at 1 week after dosing.

Pharmacokinetic Parameters of Levobupivacaine and Bupivacaine in Different Species

Species/ Strain	Sex	Route	Drug	Dose (mg/kg)	Duration (day)	Cmax (ug/ml)	Tmax (h)	AUC 0-∞ (h.ug/ml)	T1/2 elim (h)
Rat/Sprague -Dawley	M	IV	Levo.	1.0	1	0.55	0.08		
Rat/Sprague -Dawley	M F	SC	Levo.	10	1	M-1.49 F-1.31	M-0.75 F-2.0		
Rat/Sprague -Dawley	M F	SC	Levo.	10	1	M-1.10 F-0.72	M-0.75 F-0.5		
Rabbit/NZ White	F	SC	Levo.	5.0	1	P-1.1 B-0.7	P-1.0 B-0.75-2.0		
Rat/Sprague -Dawley-pregnant	F	SC	Levo. Bupi-	10 20 30 10	11	1.453 1.128 1.953 0.867	0.5 0.25 0.25 0.5	1.724 - 4.099 0.654	1.20 - 1.90 0.72
Rat/Sprague -Dawley	M & F	SC	Levo. Bupi	10 20 30 10	14	0.398 0.934 1.205 0.319	1.0 0.5 0.5 1.0	- 3.258 3.915 -	- 2.63 1.87 -
Rabbit/NZ White	F	SC	Levo Bupi	5 10 20 5	6	0.260 0.686 0.937 0.229	0.67 0.5 0.33 0.58	3.168 9.134 33.561 1.238	10.09 17.13 33.83 3.75

P - Plasma B - Blood

Comparative PK values in human following intravenous administration of 40 mg levobupivacaine or 40-mg bupivacaine (study 030302) are presented below:

Parameters	Levobupivacaine	Bupivacaine
C_{max} (µg/ml)	1.44	1.42
$AUC_{(0-inf)}$ (µg.hr/ml)	1.15	1.17
$t_{1/2}$ (hr)	1.268	1.153
Cl (l/hr)	39.06	38.12
Vd (l)	66.91	59.97
MRT (hr)	1.423	1.409
T_{max} (hr)	0.144	0.159

The pharmacokinetic and metabolic studies in humans, animals and in vitro suggest that levobupivacaine (Chairocaine^{R}) has a very similar kinetic profile to bupivacaine.

Summary and Evaluation of Reproductive Toxicology Studies

Subcutaneous treatment of male and female rats during segment I (fertility and general reproductive performance) with 30 mg/kg/day levobupivacaine induced mortalities in both sexes but had no effects on mating performance and fertility. There were no toxic effects at 10 or 20 mg/kg/day of levobupivacaine. There were sores/lesions at the injection sites in the intermediate and high dose animals.

Treatment of Crl:CD^{R} rats from Day 6 to Day 15 of pregnancy with bupivacaine or levobupivacaine at 6 or 18 mg/kg/day subcutaneously did not produce significant drug-related effects on the pregnant females, developing fetuses or any teratogenic effects. The malformations (~9/300) were incidental and showed squat foetuses (6), diaphragmatic hernia, micro/anophthalmia with retinal folding or small orbital sockets. There were no obvious differences between the racemate and the S-enantiomer.

Subcutaneous treatment of rabbits with levobupivacaine at 20 mg/kg/day from Days 7 to 19 of gestation induced convulsive episodes and deaths. No treatment-related embryo toxicity or teratogenicity was evident at the maternally toxic dose level. External, visceral and skeletal variations occurred in all groups but there was no dose-related trend in the number of foetuses or litters affected. According to the author of this study "on a dose-for-dose basis, the reaction to levobupivacaine and the comparator (bupivacaine) was similar." During toxicokinetics analysis, no R-bupivacaine was detected in the plasma after dosing with levobupivacaine. Dosing with levobupivacaine generated ~two fold higher S-bupivacaine C_{max} and AUC $_{0-t}$ values compared with an identical dose of bupivacaine.

Subcutaneous administration of levobupivacaine at 30 mg/kg/day from day 6 of gestation to day 21 postpartum in rats induced deaths in the parent's generation but no effects on the pre- and post-natal development of the F1 generation.

Intravenous infusion of levobupivacaine, bupivacaine or ropivacaine at 0.07 mg/kg/min for 15 min followed by 0.035 mg/kg/min for 45 min did not affect uterine blood flow(UBF), intra-amniotic pressure (IAP), maternal or fetal drug concentration in pregnant (125 - 135 days) ewes weighing 40 - 80 kg. The systemic toxicity of levobupivacaine, bupivacaine and ropivacaine were investigated in pregnant and non-pregnant sheep. Pregnant and non-pregnant sheep receiving bupivacaine reached convulsion and hypotension with lower doses than receiving levobupivacaine and ropivacaine. The accumulated dose and time to toxic events were generally highest for ropivacaine compared with levobupivacaine and bupivacaine. There were no differences between pregnant and non-pregnant sheep for all the three drugs tested in either the accumulated dose or accumulated time to the onset of each toxic event; convulsion, hypotension, apnoea followed by circulatory collapse.

Summary and Evaluation of Genotoxicology Studies

The S-enantiomer of bupivacaine HCL was not mutagenic in Salmonella test system in the presence or absence of metabolic activation (S-9 rat liver fractions).

Levobupivacaine did not induce structural chromosome aberrations in cultured human peripheral blood lymphocytes in the presence and absence of S-9. Levobupivacaine or bupivacaine at a dose of 45 mg/kg/day subcutaneously did not induce micronuclei in the polychromatic erythrocytes of the bone marrow of mice.

Overall Summary and Evaluation

Levobupivacaine is the S-enantiomer of the racemic drug bupivacaine (e.g.,Marcaine). Aberg, (Acta Pharmacol Toxicol 1972; 31:273-286) studied the acute toxicity of racemic bupivacaine and its enantiomers in female NMRI mice, female Sprague-Dawley rats and male albino rabbits. The results of this non-GLP study are summarized in the table below.

Species	Route of Admin.	Racemate LD_{50} (mg/kg)	Levobupivacaine LD_{50} (mg/kg)	Dexbupivacaine LD_{50} (mg/kg)
Mice	IV	7.3±1.0	9.6±1.0	7.9±1.0
Rats	IV	5.6±0.2	7.2±0.4	3.8±0.2
Mice	SC	53±5	100±9	30±3
Rats	SC	48±3	52±4	38±3
Rabbit	IV	6.9±0.7	9.7±0.8	5.5±0.3

In a GLP-study performed for the sponsor, the estimated median intravenous lethal doses (LD_{50}) for male rats were 4.35 (3.62-8.67) mg/kg and 4.97 mg/kg (range was not provided by the sponsor) for bupivacaine and S-bupivacaine, respectively. During this GLP-acute intravenous toxicity study, the deaths occurred within half an hour of the dosing. Levobupivacaine is pobably slightly less toxic than bupivacaine. Single epidural (0.2 ml/kg of 0.75%) or intrathecal (3 mg/animal) administration did not show neurotoxicity.

Levobupivacaine presented a close similarity to bupivacaine in reproductive toxicity, genetic toxicity and pharmacokinetics in animals. The results also show the local anesthetic potency of levobupivacaine (S-enantiomer) to be approximately equal to bupivacaine (racemic mixture) in several species.

Preclinical studies show that levobupivacaine may have higher cardiovascular safety profiles than bupivacaine. This is shown by the available in vivo and in vitro data. In pigs following intracoronary administration of levobupivacaine, bupivacaine or ropivacaine, ventricular

fibrillation was the cause of death for all these compounds. But the lethal dose for levobupivacaine (approx. 8 mg) was higher than bupivacaine (approx. 5 mg). The lethal dose of ropivacaine was similar to that of levobupivacaine. QRS prolongation, an index of cardiotoxicity, occurred at slightly higher doses of levobupivacaine than of bupivacaine. In sheep, infusion of bupivacaine (150-200 mg) and levobupivacaine (300-350) caused ventricular tachycardia leading to fatal ventricular fibrillation. In another sheep study, the median plasma levels that caused cardiovascular collapse were approximately 5 µg/ml for levobupivacaine and 2 µg/ml for bupivacaine. The in vitro electrophysiological and contractility data also show similar trend for less cardiotoxic potency of levobupivacaine. To substantiate safer cardiovascular and CNS profiles, this reviewer felt that additional data as recommended by the anesthetic advisory committee are required.

In response to an FDA request based on the Anesthetic and Life Support Drugs Advisory Committee recommendations of March 24, 1997, the sponsor is conducting studies to compare the direct effect of levobupivacaine and racemate (bupivacaine) on CNS and heart in conscious large animals following close intra-arterial injection. According to the sponsor, the heart-direct coronary artery infusion (CNS performance maintained) experiment is in progress. A CNS-direct carotid artery infusion (cardiac performance maintained) investigation will be performed later. A study on resuscitation after cardiovascular infusion in dogs is ongoing and will be completed by the end of this year. These data are needed to substantiate the sponsor's claims of safer cardiovascular and CNS profiles for levobupivacaine than for the racemate.

Recommendations

The pharmacological and toxicological profile of levobupivacaine obtained from the laboratory animals and nonclinical studies support the reasonable safety of this compound for the proposed use in humans This application is, therefore, approvable from the standpoint of pharmacology/toxicology, with the following amendments of the labeling as noted below:

1 Page 8 of 31 Clinical Pharmacology:

The last paragraph

should be revised to read

The data from the on-going preclinical studies (refer to page 3) should be submitted to the FDA for evaluation to substantiate that Chirocaine possesses higher cardiovascular safety

profiles than bupivacaine.

2. Page 20 of 31 Carcinogenesis, Mutagenesis, Impairment of Fertility

I [should be changed to read

Based on the available data, this is more precise and correct statement on the genotoxicity of levobupivacaine.

II [should be revised. According to 21 CFR 201.57 on the reproductive data, the multiples of the doses comparing those of the animals and humans should be included in the label either in term of AUC or body surface area unit. Accordingly, this information has been incorporated in the label by this reviewer as follows:

"Studies performed with Chirocaine in rats at 30 mg/kg (180 mg/m^2) did not demonstrate an effect on fertility or general reproductive performance over two generations. This dose is approximately one half the maximum recommended human dose (570 mg) based on body surface area (352 mg/m^2)."

3. Page 20 of 31 Pregnancy Category B

should be revised to

should be changed to

There were no treatment-related effects on late fetal development, parturition, lactation, neonatal viability, or growth of the offspring in a perinatal and postnatal study in rats at dose levels up to approximately one-half the maximum recommended human dose (570 mg or 352 mg/m^2) based on body surface area.

should be deleted.

Pharmacology Portion of Letter to Applicant:

Before the application can be approved from the pharmacology/toxicology point of view, the following revisions of the labeling should be made:

1 Page 8 of 31 Clinical Pharmacology:

should be revise to read

2. Page 20 of 31 Carcinogenesis, Mutagenesis, Impairment of Fertility

Long term studies in animals of most local anesthetics, including Chirocaine, to evaluate the carcinogenic potential have not been conducted.

Mutagenicity was not observed in bacterial mutation assay, mouse lymphoma cells mutation assay, chromosome aberrations in human blood lymphocytes, and micronuclei in the bone marrow of treated mice.

Studies performed with Chirocaine in rats at 30 mg/kg (180 mg/m^2) did not demonstrate an effect on fertility or general reproductive performance over two generations. This dose is approximately one half the maximum recommended human dose (570 mg) based on body surface area (352 mg/m^2).

3. Page 20 of 31 Pregnancy Category B

Teratogenicity studies in rats (180 mg $/m^2$) and rabbits (220 mg/m^2) did not show evidence of any adverse effects on organogenesis or early fetal development. The doses used were approximately equal to one-half the maximum human dose (570 mg/person, 352 mg/m^2) 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 a perinatal and postnatal study in rats at dose levels up to approximately one-half the maximum recommended human dose (570 mg or 352 mg/m^2) based on body surface area.

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

should be deleted.

/S/

M. Anwar Goheer

/S/

Dou Huey (Lucy) Jean

Nov. 6, 1998