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Local anaesthesia in term- and preterm infants

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LOCAL ANAESTHESIA IN TERM- AND PRETERM INFANTS

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Local Anaesthesia In Term- And Preterm Infants

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To Pär and Erik

"Caring for a preterm infant is holding a small fluttering life in your hand"

Abstract

Background

Local anaesthesia is known to be a safe and effective method for postoperative pain management in adults and children. This convenient method is however rarely used in term and preterm infants, despite its obvious benefits. This is due to lack of research and experience in the area.

The free fraction of local anaesthetics is responsible for both toxicity and efficacy. Alpha-1-acid-glycoprotein (AAGP) has a high affinity for amide local anaesthetics, thus plasma levels of AAGP determine the free fraction of LA. This is why this glycoprotein is important when determining dosages of LA infusions. There are very few studies concerning AAGP in newborns and none in extremely preterm infants.

Aims

- To assess the safety and efficacy of local anaesthesia administered by wound catheter after major surgery in term- and preterm infants.
- To investigate levels of alpha-1-acid-glycoprotein in new-borns after delivery, and their correlation to age and mode of delivery.

Methods

The studies were performed at departments of Neonatology and Paediatric Anaesthesia and Intensive care at Karolinska University Hospital. All patients were term and preterm newborn infants.

In the first two studies blood was obtained postoperatively after major surgery from patients with wound catheters for determination of Levobupivacaine (LB) concentrations after intermittent (StudyI) or continuous (Study II) infusion with LB: The first study used a sampling protocol of six samples over a 24 hour period and the second study used four samples in 72 hours. Pain was assessed using validated pain scales and morphine consumption was registered. In Study III levels of AAGP was determined in blood sampled from umbilical arteries direct after delivery.

Results/Conclusions

Studies I and II: The studied infusion regimens with LB administered by wound catheters was associated with plasma levels of LB well below toxicity. We also noted good wound healing, low pain scores and reduced need for opioids compared to our normal clinical experience. **Study III**: This study suggest a significant correlation between increasing gestational age and increasing levels of AAGP in plasma. AAGP levels in plasma seem to correspond with increasing maturity of the new-born infant. Gender and birth weight did not seem to influence AAGP concentrations in plasma. Infants born vaginally had significantly higher levels of AAGP compared to those born with planned caesarean section.

- Local anaesthesia by wound catheter administration in term- and preterm new-born infants is a safe method to use.
- Gestational age and mode of delivery should be factors when determining dosage of local anaesthetics.

List of publications

I. **Plasma concentrations of levobupivacaine associated with two different intermittent wound infusion regimens following surgical ductus ligation in preterm infants.**
Anell-Olofsson M, Lönnqvist PA, Bitkover C, Lundeberg S, Larsson BA, Eksborg S, Bartocci M
Paediatr Anaesth. 2015; 25: 711-718.

II. **Plasma levels of levobupivacaine during continuous infusion via a wound catheter after major surgery in newborn infants: An observational study.**
Krylborn J, **Anell-Olofsson ME**, Bitkover C, Lundeberg S, Bartocci M, Stiller C-O, Larsson BA
European journal of Anaesthesiology 2015; 32: 851-856.

III. **Plasma concentrations of alpha-1-acid-glycoprotein in preterm and term infants at birth: Influence of mode of delivery and implications for the use of local anaesthesia.**
Anell-Olofsson M, Ahmadi S, Eksborg S, Lönnqvist PA, von Horn H, Bartocci M
Br J Anaeth, 2018; doi.org/10.1016/j.bja 2018.01.034 (Epub ahead of print)

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List of Abbreviations

AAGP	Alpha-1-acid-glycoprotein
ALPS	Astrid Lindgren Children´s Hospital Pain scale
BII	Bolus intermittent infusion
DII	Delayed intermittent infusion
EDA	Epidural analgesia
EDIN	Echelle Douleur Inconfort Noveau pain scale
GA	Gestational age
LA	Local anaesthesia
LAST	Local anaesthesia systemic toxicity
LB	Levobupivacaine
NEC	Necrotizing enterocolitis
NICU	Neonatal intensive care unit
PICU	Paediatric intensive care unit

1 INTRODUCTION

Elsa

101 years ago Elsa was born as the first child to her parents. The lady who delivered her immediately noticed that Elsa was very small. The lady used the kitchen scale and found that Elsa weighed 2,1 kg. Her skin looked very red and shiny. She was breathing and screamed with a fragile voice but didn't have the strength to breastfeed, and as the days went by she deteriorated and got more and more tired. Elsa´s grandmother tried to feed her with diluted cows milk by spoon. Despite the efforts made to feed Elsa she passed away on the fifth day of her short life.

Patrick

53 years ago Patrick was born in gestational week 34 weighing 2 kg after a caesarean section due to bleeding and ablation of the placenta. Patrick developed difficulties in breathing and deteriorated within the first 24 hours. He was transmitted to another hospital where he was put in a hyperbaric chamber to help his condition but it didn't make his breathing more stabile. He died just two days old.

Erik

16 years ago Erik was born in gestational week 34 weighing 2 kg after caesarean section due to ablation of the placenta. Erik was directly admitted to the Neonatal intensive care unit (NICU) of the hospital. He developed difficulties in breathing and received medical attendance and professional nursing. On the second day of his life Erik was already getting better. When he was just one week old he could go home with his parents. Today he is a healthy 16 year-old boy and has not spent a day in hospital since he left the neonatal ward.

Otto

5 years ago Otto was born in gestational week 25 weighing 700 g. He immediately contracted acute lung disease due to immaturity and was admitted to the NICU were he was given ventilator treatment for one week. At two weeks of age he underwent heart and bowel surgery for conditions common for extremely preterm babies. Otto is now 5 years old, and still has to visit the hospital from time to time. He has short bowel syndrome, and he is often in need of inhalation

during the cold season. Otherwise he is doing fine and he had nice results in the 5 year follow up including psychological tests.

Hugo

6 months ago Hugo was born in gestational week 22 weighing 420 g. Being very fragile and not capable of breathing by him self he was immediately put on the ventilator and consequently needed full intensive care for several months. Hugo was operated on more than 20 times in the bowel and chest during his stay in the neonatal unit, and he also lost an arm due to bad circulation.

Hugo had major bleedings in the brain and developed severe lung disease due to his extreme immaturity. He is still on the ventilator and in need of various operations to his bowels and on his arm and accordingly in great need for advanced pain medication.

2 BACKGROUND

Caring for the woman giving birth is one of the oldest forms of organized medicine. In the ancient world, the focus of the midwife was solely on the safe delivery of the mother. However, even in pre-modern days efforts were made to help infants with different postnatal issues such as being small, not holding the temperature, having difficulties in feeding etc.

The premature birth and death of Patrick Kennedy, son of U.S. President Jon F. Kennedy, is often considered the birth of modern neonatology. In the attempt to save the presidential baby´s life, new ground was broken, uncharted medical territory was explored and a new medical discipline came to life.

The stories in the introduction show us that modern neonatology has come very far in a short time. We are now able to safe the lives of infants born as early as gestational week 22 and weighing as little as 400 g. As thrilling as these medical advances may be, one has to remember that this new category of patients invariably has a need for advanced intensive care and painful procedures. As in the example above with Otto and Hugo common procedures are tracheal intubation, intravenous and arterial puncture, insertion of central venous catheters and major surgery. Common indications for surgery are persistence of arterio-venous duct and nectrotizing enterocolitis (NEC).

It was only some decades ago that the common belief, even in the profession, was that term- and preterm infants did not have the capability of feeling and remembering pain. This view of course made any pain treatment of infants superfluous. Research and clinical experience have shown us the opposite. These patients are much more vulnerable to pain due to their immature and growing nervous system.[1-3] Pre-terms are also at great risk of developing both short- and long-term complications to pain.

Term and near term infants are also subjected to major surgery due to malformations such as gastrochisis, omfalocele, diaphragmatic hernia, oesophageal and duodenal atresia etc.

Postoperative analgesia

Postoperative pain remains one of the main problems both when it comes to patient satisfaction and delayed discharge from hospital after surgery.[4, 5]

There is further more a potential risk of developing persistent postoperative pain (PPP) which in turn could lead to chronic pain.[6]

Opioids are still the base for postoperative analgesia in both adults and children. This is despite well-known negative side effects such as constipation, unwanted sedation, respiratory depression and withdrawal symptoms. There are long-term negative effects such as immune suppression and delayed neurological development.[5, 7-10] Treatment with opioids makes the patient stay longer on the ventilator, thus prolonging the time admitted to hospital. This, in turn, causes problems in developing interaction with the parents and increasing costs for society.

Other intravenous drugs such as paracetamol and clonidine are used postoperatively alongside opioids as part of a multimodal analgesic regime.[11] Non-steroidal anti-inflammatory drugs (NSAID) are not used in new-borns.

Local Anaesthetics/Levobupivacaine

Local Anaesthetics has been used since 1884 starting with cocaine for eye surgery. Swedish researchers and physicians have a long history of developing and using local anaesthetic drugs and regional anaesthetic techniques.

We know that local anaesthetics and regional anaesthetic methods has good effects and are safe in adults and older children[4, 12-14], but are still infrequently used in neonates.

Levobupivacaine (LB) is the local anaesthetic of preference in Astrid Lindgren Children´s Hospital due to its favourable low-toxic profile and long duration. It is also the local anaesthetic used in the studies of this thesis.[15] It is the pure S (-) enantiomer of bupivacaine and is seen as a safer alternative than its racemic parent. LB is an amino-amide type of local anaesthetic drug with a more pronounced sensory- than motor block.[15] The potency of LB is similar to that of bupivacaine, although it has been shown to have longer duration in lower doses.[16-18] It has also been shown that the duration of LB blocks is much longer in peripheral and regional blocks than in epidural blocks.[19]

Mechanism of action

LB as well as other local anaesthetic blocks the generation and conduction of nerve impulses. This is accomplished by slowing the propagation of the nerve impulse and by reducing the rate of rise of the action potential.[20] The drug binds to the intracellular portion of sodium channels and blocks sodium influx into nerve cells, thereby preventing depolarization.

Onset of action of LB has been noted to be less than 15 minutes in multiple studies.[19, 21-25] LB is a long acting local anaesthetic drug with different durations in different kinds of blocks.[19]

Absorption

The plasma concentration of LB depends on route of administration and dosage. The route is important because there is a great difference of vascularity in different tissues.[15]

Metabolism

LB is metabolized by enzymes. CYP3A4 isoform and CYP1A2 isoform is mainly responsible for the metabolization to desbutyl-levobupivacaine and 3–hydroxy -levobupivacaine, respectively.[18] Desbutyl -levobupivacaine is converted to sulphate ester conjugates and glucoronic acid. It is likely that liver dysfunction have an effect that could be significant when it comes to elimination of LB (Purdue Pharma L.P 1999, Abbott laboratories 1999)

Elimination

Approximately 95% of metabolites of LB are eliminated via the urine (71%) or faeces (24%) in 48 hours. (Purdue Pharma L.P 1999, Abbot laboratories 1999)

Toxicity

There is always, as for all local anaesthetics a potential risk for LAST (Local Anaesthesia System Toxicity). LAST presents with diverse symptoms such as cardiac arrhythmias and seizures. Potential toxic levels of racemic bupivacain are 0,3 microg ml $^{-1}$ and 4 microg ml $^{-1}$ unbound and total respectively.[20, 26] LB appears to cause less myocardial depression and arrhythmias than both bupivacaine and ropivacaine, even at higher concentrations. This makes it a very good alternative when using local anaesthetics in fragile patients.[18, 27] Studies on animals and volunteers have shown that there are less CNS toxicity when using LB compared to bupivacaine or ropivacaine.[18, 28]

All data concerning plasma concentrations is either from in vitro studies, animal studies or adult volunteers.

Epidural analgesia

Epidural analgesia (EDA) has been called the "golden standard" of postoperative pain management in both adults[29] and children.[13, 30] It has been considered equally safe and effective, and thus considered to be the first choice of treatment. This preferential treatment of EDA has recently been questioned due to a suspected underestimation of the risks involved as well as an overestimation of its efficacy.[4, 31] In the paediatric population there has been reports of catastrophic outcomes when using epidurals.[32, 33] Questions concerning the duration of epidural infusions has also been raised for the neonatal population due to uncertainty regarding the rate of drug elimination.[34] Furthermore, epidurals are seldom used in preterm patients due to the obvious difficulty and risk concerning the insertion

of the catheter in the neonates narrow epidural space. In our unit sacral analgesia in neonates, are considered a both safer and easier alternative to EDAs. In our practise, a catheter is not placed in situ and the method is only used for "lower" surgery such as inguinal hernia and anal atresia.

Nerve blocks

Different kinds of peripheral nerve blocks are considered a better method than intravenous or epidural analgesia and are today more frequently used.[35, 36] With the introduction of ultrasound these techniques has become very safe and are nowadays also used in paediatric anaesthesia.

In small infants such as neonates and preterms however, the method still presents some difficulties. The small size of the patient makes it very difficult to place the catheter in the right position as well as administering correct dosage of the local anaesthetic. There is theoretically also a greater risk for intra-neural or intravascular injection of the local anaesthetic.

Local infiltration analgesia using a mix of local anaesthetics, adrenalin and ketorolac is commonly used for postoperative analgesia after knee and hip arthroplasty, with or without indwelling catheter. This technique is opioid sparing and easier to perform than for instance femoral block but it is not used in paediatric surgery.[37]

Wound catheter infusions

In the neonatal department at Karolinska hospital we have since a decade used wound catheters as the primary analgesic method after surgical closure of the arterio-venous duct in extremely preterm infants. This is mainly due to the fact that it is technically very advanced, time consuming and to some extent dangerous to insert an epidural catheter in a patient weighing around 700 g and we know that high doses of morphine and other opioids effects the preterm negatively both in the short and long term.[7-9]

The easiest and probably safest way to administer local anaesthesia is under direct vision by the surgeon in the wound and also in many cases effective and opioid sparing.[38] Avoiding neural damage and intravascular injection is important and this is more likely to happen if the patient is very small.

Wound catheter techniques have been shown to be both safe and effective with opioid sparing effects in adult postoperative care.[39, 40] There are also reports not showing an overall positive effect of these techniques.[41, 42]

The catheters are multi-orifice tubes of different lengths and diameters. These are placed in the wound by the surgeon after major surgery (***Figure 1***). The catheter is placed superficially to the muscle fascia under direct supervision of the

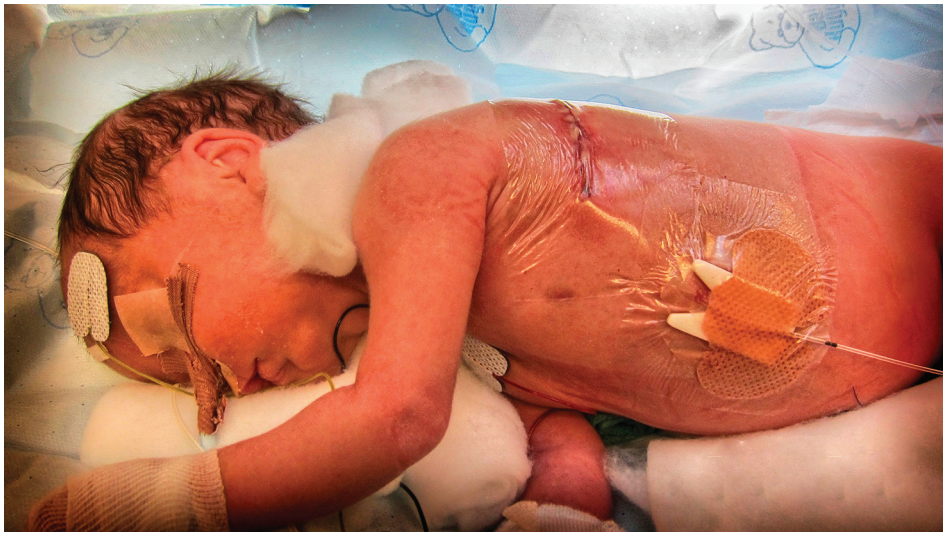


Figure 1

surgeon. Local anaesthetics are then administered through the catheter as boluses and/or as infusions postoperatively.

Wound catheters has until now been infrequently used in the paediatric population even tough there are studies showing good results including intra- and postoperative use following sternotomy, iliac bone grafting and minor to moderate abdominal surgery.[43-45]

Due to its simplicity and safety, wound catheter infusions might be a perfect way to introduce postoperative analgesia treatment with local anaesthesia in neonatal patients.

Pain scoring in neonatal patients

There are for obvious reasons great difficulties in assessing pain in the neonatal population with the patients not being able to communicate and also being neurologically immature. The response to painful procedures is very different in an extremely preterm infant compared to a term new born. For these reasons there are no universal approach to neonatal pain assessment.[46] Objective measurements such as blood pressure, heart rate, respiratory rate are used alongside with validated pain scales. In the studies in this thesis we have used the scales that were "clinical standard" on the wards at that time. EDIN (Echelle Douleur Inconfort Noveau).[47] and ALPS (Astrid Lindgren Pain Scale).[48] Both of these scales are behavioural markers. Facial expression, body movement, quality of sleep, contact with staff and consolability are examples of variables being assessed. Scores are given (0-3, 0-2 respectively in the different scales), and when the score reaches a certain figure, interaction of some sort must be done to relieve the infants pain.

Alpha-1-acid-glycoprotein

Alpha-1-acid-glycoprotein (AAGP) is an acute phase plasma protein consisting of 41% carbohydrate and 59% protein.[49, 50] AAGP is produced by hepatocytes, but extra-hepatic production by endothelial cells has been described.[49, 51]

Two genes located on chromosome 9 (ORM1 and ORM2) have been identified to encode two types of AAGP.[52] The two types of AAGP differs by 22 amino acids.

The only established function of AAGP is to act as a carrier of basic and neutrally charged lipophilic compounds such as basic drugs (for example local anaesthetics).[49] AAGP is alongside with albumin and lipoprotein the major drug binding protein in plasma.

AAGP is produced and released in response to different types of stress such as inflammation, trauma or surgery.[51, 53] The acute phase reaction causes cytokines, prostaglandine E2 and cyclic adenosine monophosphate (cAMP) to stimulate the production of AAGP. Cancer, malnutrition and inflammatory disease such as rheumatism can also affect the production of AAGP.[49, 53] The levels of AAGP may also vary due to non-pathological factors such as weight, pregnancy, age, tobacco smoke etc.

As mentioned above LA has a high affinity for AAGP thus altering the free fraction, which is responsible for both toxicity and efficacy of the drug. It is already well known that AAGP levels alter due to surgery thus affecting the free fraction of the administered anaesthetic. Since AAGP is produced in the liver, the function and maturity of the organ also has great bearing on the dosage of local anaesthetics.

There are studies showing that new-borns have much lower levels of AAGP than adults[54] Prior to our studies there were no data on the influence of mode of delivery on AAGP levels.

3 AIMS

This project aimed to improve and gain knowledge of local anaesthesia in term- and preterm infants.

1. To investigate if local anaesthesia is a safe and effective method in term and preterm infants when used via a wound catheter after major surgery.

2. Whether the plasma concentration of LA in neonates reaches levels linked to toxicity using a wound catheter with levobupivacaine.

3. To search for signs of negative effect of wound healing

4. To determine normal values of the plasma glycoprotein alpha-1-acid-glycoprotein(AAGP) in term and preterm infants, and look for differences due to gestational age, birthweight, gender and mode of delivery in the levels of AAGP.

4 METHODS

The studies were performed according to the declaration of Helsinki and approved by the Regional Ethical Review Board of Stockholm, Sweden.

Parental consent was obtained in all cases.

Patient Demographics

108 new-born infants were included in the studies.

Study I Following Ethics Committee (Dnr: 2008/482-31) approval and written parental informed consent, 18 extreme preterm infants scheduled for surgical closure of patent ductus arteriosus (PDA) were included into the study. (***table 1***) Median weight was 721g and the infants were born between gestational week 23 to 27. The studied groups were similar in terms of patient demographics.

Group	**BII**	**DII**
Gender (M/F)	6/3	4/5
Gest age at birth (weeks)	24.8 (24.0 - 26.6)	26.0 (24.4 - 29.4)
Age at op (weeks+days)	28.4 (26.0 - 32.0)	29.3 (26.8 -33.3)
Birthweight (g)	700(564 - 937)	752 (600 - 1189)

Table I *BII= Bolus intermittent infusion, DII= Delayed intermittent infusion*

Study II Following Ethics Committee (Dnr: 2007/1462-31) approval and written parental consent, 20 new-born infants scheduled for major surgery (thoracic or abdominal) were included into the study (***table 2 and 3)***. All patients were less than 1 month of age at inclusion.

Diagnosis	**Patients**
Colonic atresia	1
Congenital diaphragmatic hernia	5
Cyst of the lung	3
Duodenal atresia	3
Duodenal stenosis	1
Malrotation of the Intestine	1
Oesophageal atresia	5
Tracheoesophageal fistula	1

Table 2 *Diagnosis*

Patients	
Sex (M/F)	14/6
Gest. age at birth (weeks+days) median (min-max)	39+6 (36+4-41+5)
Postnatal age at surgery (days) median (min-max)	4 (1 - 30)
Weight (g) median (min-max)	3480 (2870 - 4290)

Table 3 Patient demographics

Study III Following Ethics committee (Dnr: 2014/64-31/2) approval and written parental consent 70 new-born infants were included into study III (table 4). The infants were born between gestational week 27 to 41.

Mode of delivery	**Vaginal**	**Elective caesarean section**	**Acute caesarean section**
Number of infants (n)	31	26	13
Gender (M/F)	17/14	16/10	7/6
Gestational age at birth			
(days) median	273	265	263
(min-max)	(191-295)	(222-279)	(190-293)
Body weight (g)			
median	3363	3439	2873
(min-max)	(1734-4630)	(1234-4790)	(1058-4000)
AAGP concentration			
(g L^{-1}) median	0.189	0.110	0.182
(IQR)	(0.142-0.263)	(0.094-0.157)	(0.121-0.376)

Table 4 Patient demographics

Anaesthetic methods

Study I The patients were already intubated and mechanically ventilated when entering the operation room and had arterial and venous lines in place. Premedication was never used. All infants received atropine (10 microg kg -1), fentanyl (10 microg kg -1) and atracurium (0.5 mg kg -1) followed by general anaesthesia maintenance with sevoflurane 1.0-1.5% in oxygen/air.

Additional boluses of fentanyl and atracurium were given as needed during the operation. A maintenance infusion of glucose 2.5% was given throughout the procedure at an infusion rate of 5-10 ml kg^{-1} h^{-1}. If intraoperative hypotension occurred bolus injections of albumin (5%) were given in doses of 5-10ml kg^{-1}. Hypotension was treated at a reduction of mean arterial pressure with more than 20% compared to preoperatively.

Study II No premedication was used. The majority of the patients were intubated at the operation room whereas a few were already intubated in the intensive care unit. To facilitate endotracheal intubation all patients received atropine (10-20 microg kg^{-1}), fentanyl (1-2 microg kg^{-1}) and thiopentone (6-8 mg kg^{-1}) or propofol (2-3 mg kg^{-1}) followed by succinylcholine (2-3 mg kg^{-1}). General Anaesthesia was maintained with sevoflurane 2-4% in oxygen/air. As in study I fentanyl and atracurium were given during the operation as needed.

Wound catheter placement

Study I and II Before closure of the wound the surgeon placed a wound catheter with multiple wholes at the end of it superficial to the fascia of the muscle layers. The catheters in both studies were of the same brand (Pain Buster, I-flow corporation, Lake Forest, CA, USA) and were placed under direct supervision by the surgeon. An introducer needle was fed subcutaneously from the medial angle of the wound to a point approximately 2-3 cm away (from the medial edge of the incision) and the catheter was inserted and placed alongside the length of the wound.

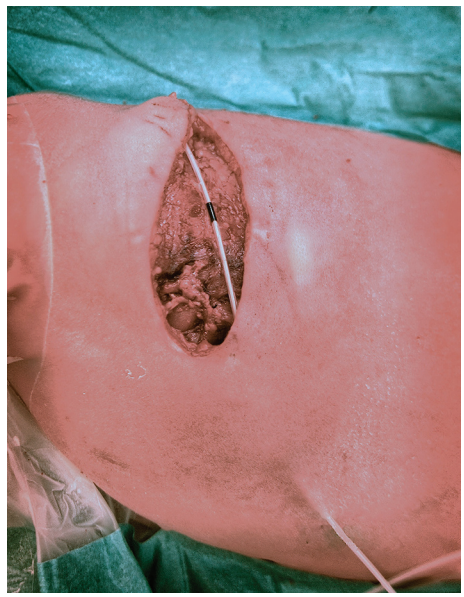


Figure 2

The size of the catheter was 19 gauge and the length 2.5 or 6.5 cm depending on the length of the incision.

After the placement of the wound catheter, subcutaneous tissue was closed with single sutures and finally the skin was closed with intracutaneous suturing technique. A transparent dressing was put over the wound as shown in ***Figure 3.***

The catheters were kept in situ for 72 hours in both studies, and then gently removed by simply pulling them out.

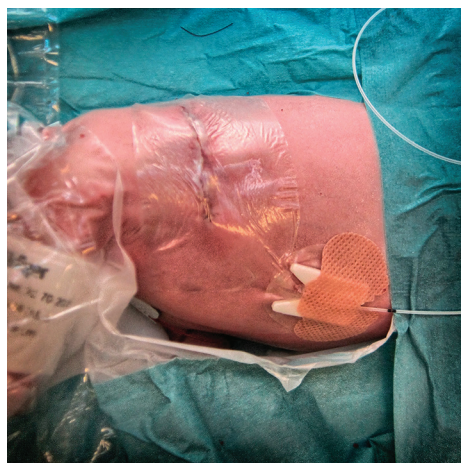


Figure 3

Dosing of levobupivacaine (study I, II)

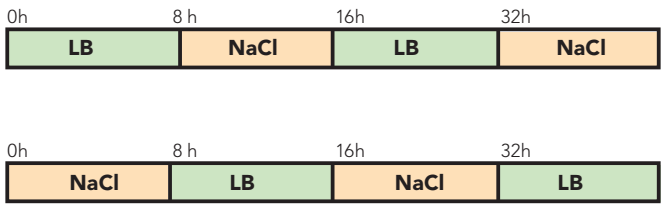
The catheters (0.08ml) were prefilled either with LB or saline according to the protocol of the study.

Study I Directly after the wound catheter was put in place by the surgeon a bolus dose was given manually with LB (Chirocaine®) 0.625 mg ml $^{-1}$ (0.2 mg kg $^{-1}$ = 0.32ml kg $^{-1}$.) in group ***BII*** and with saline in group ***DII***.

After the infant had been transported back to the neonatal intensive care unit an infusion was started according to study protocol.

Bolus plus early start of an intermittent infusion (***BII***): The infusion was started with LB (Chirocaine®) 0.625mg ml $^{-1}$ (2 mg kg $^{-1}$ 24 h $^{-1}$) and after 8 hours switched to saline (Nacl 9mg/ml). The infusion was then switched every 8 hours between LB and saline.

Delayed start of intermittent infusion (***DII***): The infusion was started with saline, given by the same rate as LB in the BII group and then changed after 8 hours to infusion with LB, then switched every 8 hours as seen in the diagram below.



Study II When the operation was finished and the wound was closed the surgeon gave a bolus dose of LB (Chirocaine®) 1.25 mg ml $^{-1}$, 0.5 mg kg $^{-1}$ (0.4ml kg $^{-1}$) through the wound catheter. When arriving at the Paediatric Intensive Care unit 20-30 minutes later a continuous infusion was started with LB (Chirocaine®) 1.25 mg ml $^{-1}$, at a rate of 0.2 mg kg $^{-1}$ h $^{-1}$ (0.16 ml kg $^{-1}$ h $^{-1}$). The intended duration of the infusion was 72 hours.

Blood sampling

All blood sampling was coordinated with other blood tests according to clinical needs. No extra scin punctures were needed in any of the patients. The 3 % rule was used in the smallest patients, meaning that the blood collected during the study never exceeded 3% of the blood volume of the patient.

Study I Blood was drawn from already existing indwelling catheters in the patients. All samples were drawn from peripheral arterial lines except in one patient where samples were drawn from a central venous line. Samples of maximum 0.8 ml were drawn at 10, 13, 16, 19, 22 and 24 hours after the bolus injection

with either saline (DII) or LB (BII) had been given by the surgeon in the operation room. Immediately after the blood was drawn from the patients it was centrifuged for 10 minutes at 4000 rotations per minute. The plasma was then pipetted and transferred into a preservative free tube where after it was frozen at -18° C until analysis.

Study II Blood was collected from already existing catheters (venous or arterial). Samples of maximum 1.5 ml were drawn at 12, 24, 48 and 72 hours postoperatively after the first bolus injection had been given in the operation room. The blood was immediately centrifuged for 10 minutes at 3000 rotations per minute and plasma was separated and frozen at – 20 ° C until analysis.

Study III Blood was sampled from the umbilical artery immediately after delivery. The blood was immediately sent to the laboratory in EDTA tubes were it was centrifuged and frozen and subsequently analysed (see under analysis).

Laboratory analysis

All analysis were performed at the Karolinska University Hospital Laboratory, Department of Clinical pharmacology and Division of Clinical Chemistry, Stockholm Sweden and performed according to Good Clinical Practice (GLP).

Study I Analysis of total plasma LB were performed using detection by a two-step liquid-liquid extraction followed by liquid chromatography-mass spectrometry (LC-MS) with doxepin as internal standard. Range of quantification of the method was 0.014-2.900 microg ml $^{-1}$. Method uncertainty was 10% (coefficient of variation).

Study II Total LB was analysed as above. In addition free unbound LB was analysed in this study with the same method. AAGP (Alpha -1-acid –glycoprotein) was analysed using nephelometry with range for quantification being 0.35 to 108 g L $^{-1}$.

Study III In the laboratory the microcuvettes were centrifuged in 20 ° C for 10 minutes with the Sigma 1-14K (LABEX instrument AB, Osterode am Harz, Germany). Plasma AAGP (orosomucoid) was analysed in EDTA plasma with an immunoturbedimetric assay on the Cobas C system (Roche Diagnostics, Germany). Lower limit of quantification was 0.1g L $^{-1}$, defined as three standard deviations above the lowest standard. Total coefficient of variation was 5 %.

Pain management and pain scoring (study I,II)

Validated pain scales for neonates were used in the studies, EDIN (Echelle Doleur Inconfort Noveau) and ALPS (Astrid Lindgren Pain Scale).

Study I An infusion with morfin at the rate of 10 microg kg h $^{-1}$ was started when the patients arrived at the NICU postoperatively. Pain was assessed every

hour by an intensive care nurse using the EDIN pain scale. Additional assessments were made as needed if the patient seemed to be in pain or if for example X ray or suctioning of the trachea was performed. According to study protocol, a pain score of 3-5 was set as a target range with min score being 0 and max 15. A score below the target range e.g. <3 for six consecutive hours resulted in a reduction of the morphine infusion rate by 2.5 microg kg $^{-1}$ h $^{-1}$. A pain score above the target range resulted in administration of recue analgesia (fentanyl 1 microg kg $^{-1}$). If the pain did not subside after the fentanyl dose the morphine infusion rate were increased by 2.5 microg kg $^{-1}$ h $^{-1}$.

Study II Pain was assesses due to PICU protocol every 6th hour using the validated Astrid Lindgren Pain Scale (ALPS). The study patients were treated with the established pain medication protocol for the unit. This includes supplementation of regional anaesthesia with paracetamol (iv) in the doses of 15-20 mg kg $^{-1}$ three or four doses per 24 hour and Clonidine 1 microg kg $^{-1}$ three to four doses per 24 hours or as an infusion 5 to 8 microg kg $^{-1}$ 24 h $^{-1}$. Opioids were given as needed for example at high pain scores. All patients were assessed and treated individually.

Signs of local anaesthesia toxicity

Signs and symptoms of LAST were carefully monitored for. All patients had ECG electrodes to detect arrhythmias and nursing staff was specifically instructed to note any signs of focal or generalized seizures.

Wound healing

During the study period all patients were examined on a daily basis and before discharge from the hospital by the surgeon with focus on wound healing. Any signs of leakage or infection were noted by the surgeon and by the nursing staff. Normal wound healing was defined as a scar, free from signs of infection or inflammation at 10 days postoperatively.

Statistics

Study I Mann- Whitney U -test was used when calculating differences in terms of plasma concentrations of LB between the two groups BII and DII . Fisher´s exact test was used when comparing classified data from two independent populations. Plasma concentration data were always expressed as median values and range (min-max) as were pain scores and morphine consumption data.

Randomization and blinding procedures: The randomization was performed by the hospital pharmacy. The patients were randomized into one out of two groups BII and DII. The randomization was performed in blocks of 10 patients. All randomization codes were derived from computer –generated numbers and

allocated using the sealed opaque envelopes techniques. The pharmacy then provided syringes that were labelled with a patient number and in which order they were to be given to the study patient. Every second syringe contained LB or NaCl 9mg/ml. The nurse caring for the patient changed the syringe according to the protocol every 8 hour.

Study II Plasma concentration data were expressed as median values and range (min /max). Pain scores were also presented as median values. The 95% confidence intervals were calculated as stated by Ott and Mendenhall.

Study III Data of AAG levels were all expressed as median values and interquartile range. Kruskal-Wallis test (non parametric ANOVA) was used when comparing levels of AAGP in infants born with different mode of delivery. Spearman correlation test was used when showing the correlation between levels of AAGP and gestational age.

Statistics used in the studies were evaluated with Graph Pad Instat 3.10.

5 RESULTS

Study I As described in "Methods" there were two groups, BII and DII with 9 patients included in each group. One patient from each group was excluded from the pharmaco-dynamic part of the study due to violation of the morphine infusion protocol.

Study II One patient was excluded due to problems with drawing blood. In three of the patients single samples were missing, these patients remained in the study.

Study III Six subjects were excluded due to missing values from the laboratory.

Plasma concentrations of Levobupivacaine (Study I)

The plasma concentrations of LB were different in the two studied groups. Figure 4 Plasma concentrations were higher in the BII group during the 10 to 16 hour postoperative period. 16-24 hours postoperatively the concentrations in the two groups converged. Maximum plasma concentration of LB in the BII group was 1.682 microg ml^{-1} as opposed to 0.549 microg ml^{-1} in the DII group. Median plasma concentrations of LB at 24 hours were 0.342 microg ml^{-1} in the BII group and 0.106 microg ml^{-1} in the DII group. There was a notable difference in LB plasma concentrations between the groups although not statistical significant (p=0.0653).

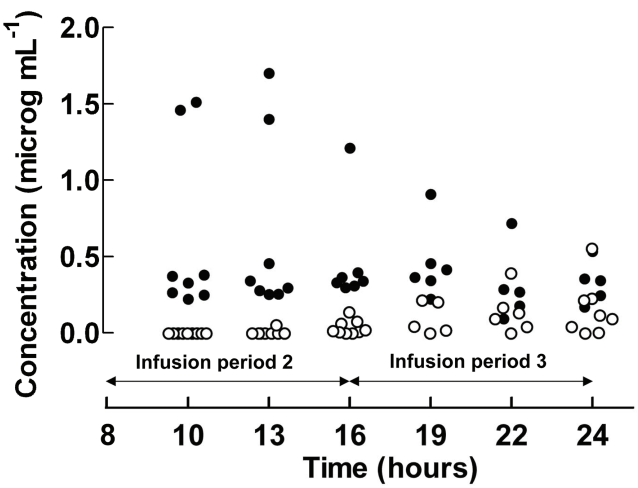


Figure 4
Plasma concentrations of levobupicacaine during infusion periods 2 and 3.
● = Concentrations in Group BII;
o = Concentrations in Group DII
During the infusion period 1(0-8h) patients in Group BII were treated with LB and patients in Group DII were treated with saline. No sampling data were obtained during this period.
During the infusion period 2 (8-16h) patients in Group BII were treated with saline and patients in Group DII were treated with LB. Sampling times: 10, 13 and 16h.
During the infusion period 3 (16-24h) patients in Group BII were treated with LB and patients in Group DII were treated with saline. Sampling times: 19, 22 and 24h.

Plasma concentrations of Levobupivacaine (Study II)

Total plasma concentrations

The maximum total plasma concentration of LB in the study was 3.15 microg ml $^{-1}$. Individual data for the patients are shown in Figure 5. Median total plasma concentrations of LB were 0.80 (0.17-1.92), 1.52 (0.06-2.05), 1.37 (0.14-3.15) and 1.30 (0.08-2.68) microg ml $^{-1}$ at 12, 24, 48 and 72 hours respectively after the bolus injection. LB concentrations tended to increase during the first 48 hours followed by a slight decrease.

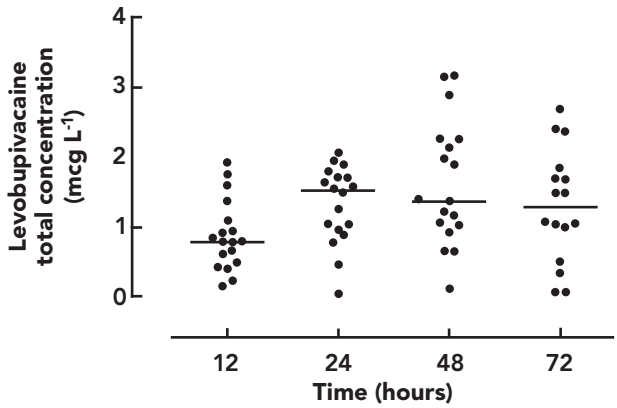


Figure 5 Total plasma concentrations of LB during the study period.

Unbound plasma concentrations

The maximum unbound plasma concentration of LB in this study was 0.210 microg ml $^{-1}$. Individual data for the patients are shown in Figure 6. Median unbound plasma concentrations of LB were 0.014 (0.02-0.05), 0.026 (0-0.059), 0.027 (0-0.208) and 0.017 (0-0.084) microg ml $^{-1}$ at 12, 24, 48 and 72 hours respectively. This shows us that opposed to the total plasma concentrations the unbound fraction seems to be more stable over the entire 72 hour period.

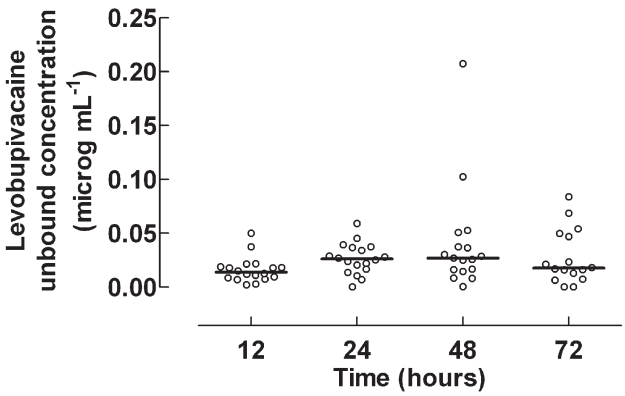


Figure 6 Unbound plasma concentrations of LB during the study period.

Plasma concentrations of Alpha-1-acid-glycoprotein (Study II)

Data for the individual patients are shown in Figure 7. AAGP was analysed preoperatively and 72 hours postoperatively. In all but four patients the concentrations increased, but no correlation was seen between the AAG concentrations and the unbound fraction of LB in this material.

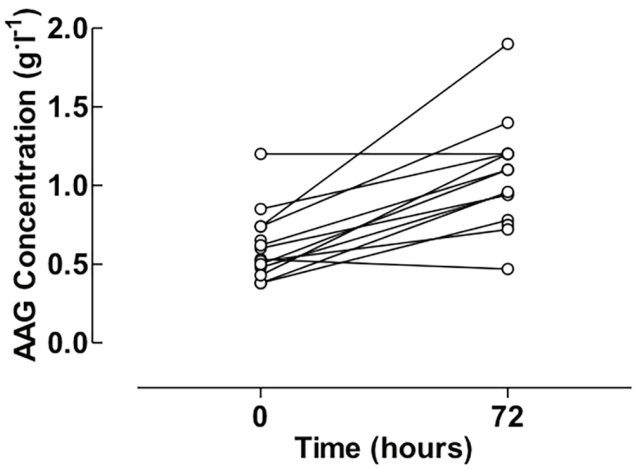


Figure 7 AAGP concentrations preoperatively and 72 hours postoperatively respectively.

Plasma concentrations of Alpha-1-acid-glycoprotein (Study III)

Median plasma concentration of AAGP in the subjects was 0.158g L^{-1} (range 0.029-0.950 g L^{-1}).

Median plasma concentrations of AAGP were 0.148 g L^{-1} and 0.160 g L^{-1} in male and female new-borns respectively (p=ns).

No correlations were found between AAGP concentrations and birth weight (r_s=0.18; p=0.162).

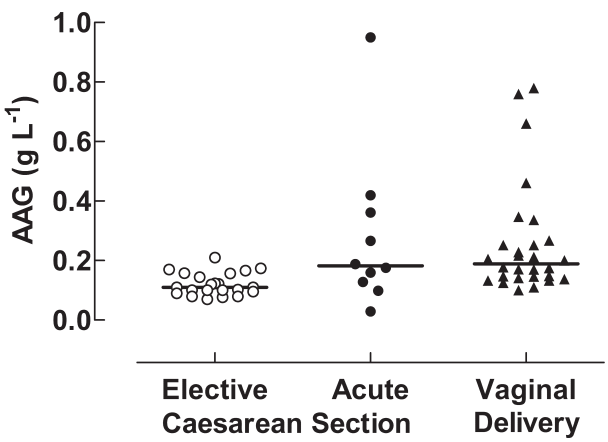


Figure 8 AAGP concentrations in relation to mode of delivery

A significant difference in AAGP concentrations were seen when comparing infants born vaginally with those delivered with planned caesarean section. Concentrations of AAGP were much higher in the vaginally born group. Median plasma concentrations of AAGP were 0.115 g L (range 0.070-0.210 g L^{-1}) and 0.189 g L^{-1} (range 0.100-0.780 g L^{-1}) in infants delivered with planned caesarean section and vaginally respectively (p=0.0003). ***Figure 8***

The concentrations of AAGP also increased significantly with gestational age of the subjects, p=0.011 in the group born vaginally. ***Figure 9***

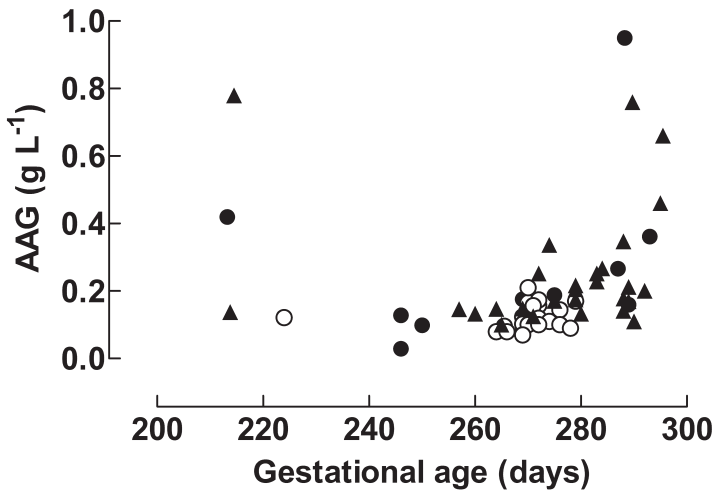


Figure 9 AAGP concentrations in relation to gestational age.

Pain assessment and treatment

Study I Pain scores were extremely low in the two groups BII (Bolus group) and DII (Delayed infusion group). Median EDIN scores were 0 and 1 in the BII and DII groups respectively. (p= ns) Numbers of patients in need for rescue analgesia (fentanyl) were significantly higher in the DII group: n = 7 as compared to n= 2 in the BII group (p=0.041). There was no significant difference in morphine consumption between the groups.

Study II Pain scores were low in all of the patients during the 72 hour postoperative period.

Hours (postop)	**12**	**24**	**48**	**72**
ALPS score (median)	**2**	**1**	**0**	**1**

The amount of opioids administered during the first 72 hours postoperatively ranged from 0 to 1.40 mg kg^{-1}. Three of the patients did not need opioids at all.

Wound healing

In Study I, a minimal leakage was noted under the transparent dressing in 8 patients. In all but one patient the wound had healed as expected 10 days postoperatively. In one patient there was signs of wound infection and also a positive culture for staphylococcus and enterococcus on day 15 after surgery.

Study II Seven infants out of the 20 included (35%; 95% CI 15.4 to 59.3) had a mild erythema around the wound that was noted during the time of infusion with LB. A leakage was noted at the edges of the wound or at the catheter insertion site in five patients. In one of those patients, this leakage was excessive and the infusion was stopped prematurely at 68 hours. In 18 patients (out of 20) the wound had healed 10 days after surgery. One patient had a wound infection with staphylococcus aureus that was successfully treated, and one patient had a local reaction around the wound due to a protruding suture.

Signs or symptoms of Local Anaesthesia Toxicity (LAST)

No signs of arrhythmias or seizures were noted in any of the patients in Study I and II.

6 DISCUSSION

Recent medical advance have made it possible to rescue extremely preterm infants to life.[56-62] These patients are invariably in need of major surgery and advanced intensive care.[63] Postoperative analgesia is for many reasons of utmost importance in this very vulnerable patient category. Both the stress of being in pain and the side effects from drugs given to treat pain can in many ways be harmful to the immature patient.

When it comes to postoperative analgesia in term- and preterm new-borns, paracetamol,[64] clonidine[11] and opioids are still used in high doses. Opioid side effects such as respiratory depression and reduced bowel motility are everyday problems in neonatal wards of today leading to prolonged ventilator treatment and increased risk of BPD. Nutritional problems and depressing effects on the immune system are other examples of opioid adverse effects in the neonatal population.[1-3, 7-9, 65, 66]

Since many years, local anaesthetic techniques have been routine in adults due to its effectiveness and safety.[13, 29, 67] The introduction of LA has lowered the need for systemic opioids which in turn has led to lesser side effects, decreased costs and shorter hospital stays.[4]

The aim with this thesis was to look further into pain treatment postoperatively in neonatal patients with focus on the use of local anaesthesia.

When determining the best way to administer local anaesthesia in neonates there are several aspects to consider such as safety, efficacy, accessibility and costs.

Epidurals and perineural techniques has been mentioned in the background part of this thesis and does not seem to be the "perfect" method in this patient category due to technical difficulties and risks for side effects.

Wound catheters have been used in adults with both convincing,[4, 39] and less convincing results.[41] On the other hand the methods seems to have many of the characteristics that we are looking for, and there are reasons to believe that wound catheter techniques could have better possibilities to work in neonates:

√ Tissues easier to penetrate due to less subcutaneous fat and thinner tissue layers.

√ Smaller incisions giving possibility for the local anaesthetic to penetrate better vertically.

√ Easy as well as safe to put into place.

Our hypothesis was that wound catheters could be a safe and effective alternative to epidurals in this patient category.

AAGP is manly responsible for binding amide local anaesthetics. We were therefore interested in looking further into AAGP concentrations in new-borns and how it correlate to different factors such as gestational age, mode of delivery, gender and birth-weight. This knowledge can be useful when determining a safe dosage of local anaesthetics in new-borns.[49, 50]

Main findings

In the two first studies of the thesis, we found plasma concentrations (both total and free) of LB to be far from potential toxic levels. Furthermore, we could not see any signs of LAST such as seizures or arrhythmias.

The main findings in study III was that AAGP plasma concentrations in new-borns are influenced by gestational age, a fact that to some extent was already known,[54, 68] we also found that the concentrations alters significantly with mode of delivery.

Furthermore, the study showed that infants delivered by planned caesarean section had significantly lower levels of AAGP than those delivered vaginally. These are new findings, and we believe they can contribute toward developing safe dosages of local anaesthetics in new-born infants. Other new findings were that we could not detect any differences in AAGP concentrations related to gender or birth weight.

The result from our Study III not only differs from former studies but also contributes with new information. We included as many as 70 new-borns in our study, this is a large cohort compared with former studies.[54, 67] Earlier studies had shown that AAGP concentrations in new-borns were 50% of adult levels.[68] Our study shows figures around only 20% of adult levels. In comparison with earlier studies our study protocol gives more accurate values for AAGP concentrations. We obtained blood samples directly after delivery from the umbilical artery, whereas previous studies have obtained samples at different times and from different sites.

Pharmacokinetics

In Study I there was a tendency of difference between the groups although not significant. The bolus and early infusion group (BII) showed higher median concentrations of LB. An interesting finding was that the maximum concentration of LB in the BII group was three times as high as in the delayed infusion group [69]. The median LB concentration in the BII group was expected to be higher especially during the first period, but the maximum concentration being so much

higher was somewhat unexpected. In conclusion, both infusion regimens created concentrations well below toxic levels but in the BII group the levels were closer to toxicity.

Both total and free concentrations of LB were measured in Study II giving us a nice complement to our first study. The concentrations in this study were found to be far below toxicity levels, a finding in line with Study I. Median LB concentrations after 24-hours of infusion were, as expected, much higher in Study II compared to Study I. This is due to the fact that there was a continuous infusion of LB, with no periods of saline infusion.

The free median concentration of LB at 24 hours in Study II was remarkably low. It was also constant during the whole study period as opposed to the total concentration, which rose from 12 to 48 hours. We have hypothesized that this could be due to a rise in AAGP, but we could not show that in this study.

In the light of these results we find LB infusions via wound catheters given postoperatively in the used doses and manner is a safe method of analgesia in these patients.

Postoperative analgesia and pain scoring

Objective measurement of pain is very difficult in any patient category.[70, 71] In new-borns, who are not able to communicate and have a different pain response pattern compared to older patients, pain scoring is even more challenging.[72-77]

In both studies we could notice less consumption of morphine compared to what usually is the case in similar situations without wound catheter analgesia. However, we could not make any significant conclusions, mainly due to the study setup (few patients, no control group).

When comparing the two studies, one can see a big difference in morphine consumption with much less need for additional opioids in Study II (performed at the PICU). This is surprising, since the protocol of study II did not include a morphine infusion. Study II was performed in the PICU, where patients from this category normally have an epidural catheter postoperatively. The subjects of Study I were patients from the NICU, where EDAs are never administered. This difference in pain management routines is a possible cause for the difference in morphine consumption between the studies. Another reason could be that it is harder to assess pain in extremely preterm than in term infants making the staff more prone to give extra opioids.

Wound healing and potential adverse effects of wound catheters

When using wound catheters there are for obvious reasons concerns about whether

healing is affected. Another potential adverse effect is infection.

These questions have been addressed in adults, and there are no convincing evidence to support increased risk of infection or delayed wound healing.[78-80] However, studies concerning wound healing are few and none of those include long-term follow up of wound healing.[81] There are multiple in vitro and animal studies that suggest that wound catheter analgesia has adverse effects on wound healing, especially at the first two stages (inflammatory and granulation) of healing. On the other hand there are also studies suggesting that local anaesthetics possesses both bactericidal and bacteriostatic effects.[82]

Our study differs from older studies mainly because of its primary focus on wound healing. We inspected the wounds on a daily basis, as well as before discharge. We also had a time limit of ten days for expected wound healing. Only two patients (one in each study) had positive wound cultures and delayed healing. One patient had a local reaction due to a protruding suture.

In our studies we saw a mild erythema in several of the patients, which disappeared shortly after catheter removal. This could be due to vasodilation as a part of an inflammatory response.

In contrast to what is seen in adult patients, we had some problems with leakage from either the wounds or from the catheter insertion sites. This is probably due to the very small incision site, and also to the greater risk of catheter dislocation compared to larger patients.

According to our clinical experience and to the findings of these studies, local analgesia by wound catheter does not affect wound healing, nor does it increase the risk of infection. We have not been able to make any statistically significant conclusions since there was no control group in the study, and since the cohort was too small.

Implications of AAGP concentrations for dosage of local anaesthetics in new-borns

The current routine for paediatric administration of LA includes dose reduction when given to neonatal patients compared to older children, and this practice is supported by our findings. Further reduction of the dose from 36 to 27 gestational weeks of age seems however unnecessary, since the normal levels of AAGP are reasonably stable in this age group. Our study also suggests that infants can produce AAGP as a response to stress (vaginal delivery). The highly significant finding that AAGP levels are higher in new-borns delivered vaginally compared to by caesarean section suggests that giving slightly higher doses of LA in the first postnatal period to the vaginally delivered infants might be a prudent course of action.

Future perspectives

Wound catheter infusion with local anaesthetics

In order to answer the question whether wound catheter infusions with local anaesthetics can replace epidural analgesia and intravenous opioids as the new "gold standard" after major surgery in neonates, there are several actions that need to be taken:

√ Clinical trials comparing wound catheter analgesia with epidural analgesia with respect to both safety and efficacy. Theses trials should also include long-term follow- up of wound healing.

√ Refining the method by developing new, softer in-wound catheters.

√ A general discussion regarding whether amide local anaesthetics should be the first choice in these patient categories is needed. Chlorprocaine, an ester local anaesthetic agent, rapidly metabolized by plasma esterases could be an interesting alternative in the neonatal group.[83]

√ Further studies of the use of adjuvants to the infused local anaethetic. Clonidine is a highly relevant option, and is already used to some extent.[84]

Alpha-1-acid-glycoprotein

There are still many questions to be answered when it comes to AAGP and newborns.

√ How does normal levels change during the first days and weeks of life?

√ Can term- and preterm infants respond to surgical trauma by producing higher concentrations of AAGP as seen in adults.

Our group will contribute with more studies in both of these fields. Hopefully we will soon be closer to introducing local anaesthesia for use in a larger number of indications, even in the most immature population, the extremely preterm infants.

7 CONCLUSIONS

1. Levobupivacaine used via a wound catheter after major surgery in term and preterm infants is a safe method. No adverse effects where noted in our studies. Local anaesthesia as used in our studies shows a favorabel profile when it comes to effectiveness and good analgesia. Morphine consumption tended to be low and pain scores were also in the low range.

2. The plasma concentrations of levobupivacaine in both studies were well below the thought toxic threashold as measured in adults and animal studies, and the unbound fraction was especially low. No signs or symptoms of local anaesthesia toxicity (LAST) were noted.

3. We could not detect any negative effect on wound healing compared to our normal clinical experience.

4. A correlation between gestational age and AAGP levels were found with increasingly higher levels in the more mature infants. Significantly higher levels of AAGP were noted in infants born vaginally compared to those born with planned caesarean section. No differences were detected in AAGP concentrations in correlation to gender and birthweight.

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ORIGINAL ARTICLE

Plasma concentrations of levobupivacaine associated with two different intermittent wound infusion regimens following surgical ductus ligation in preterm infants

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What is already known

- Local anesthetics administered by wound catheter are safe and effective in adults and older children.

What this article adds

- Our study shows that extremely preterm infants can benefit from local anesthetic infusions via wound catheters postoperatively.

Implications for translation

- Wound catheters with levobupivacaine infusion can be safe and effective in extremely preterm infants as postoperative analgesia. Regional techniques such as local anesthesia infusion via wound catheters can reduce the need for opioids and be a safe and easy alternative to epidurals in this vulnerable patient category.

Keywords
local anesthesia; pain; patent ductus arteriosus; preterm infants

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Summary

Background: Administration of local anesthetics by a surgically placed wound catheter has recently been shown to reduce the need for postoperative morphine administration in extremely preterm infants undergoing ductus ligation. The primary aim of this randomized safety study was to define the plasma levels of levobupivacaine (LB) following two different intermittent infusion regimens.

Methods: Eighteen preterm infants 23–27 gestational weeks, median birthweight 721 g scheduled for ductus ligation were included in the study. All patients were anesthetized according to a standardized protocol based on high-dose fentanyl (25–50 $\mu g \cdot kg^{-1}$). Before skin closure, a subcutaneous catheter was inserted into the wound. The patients were randomized to receive one of the two intermittent infusion regimens: Group BII: Initial bolus plus early start of the intermittent infusion or Group DII: No bolus plus delayed start (8 h) of the intermittent infusion. Blood samples for determination of LB plasma concentrations were obtained on six occasions during the 24-h postoperative observation period, as well as hourly postoperative pain assessments using the Echelle Douleur Inconfort Noveau (EDIN) pain scale.

Results: Plasma concentrations of LB ranged from 0.094 to 1.682 $\mu g \cdot ml^{-1}$ and 0 to 0.549 $\mu g \cdot ml^{-1}$ in group BII and DII, respectively. Both regimens were associated with low postoperative EDIN pain scores (24 h median of 0

and 1 in group BII and DII, respectively). No signs of systemic local anesthetic toxicity were noted.
Conclusions: The two studied intermittent infusion regimens were associated with plasma levels below potentially toxic levels and were both associated with adequate postoperative pain scores.

Introduction

Continuous thoracic epidural or paravertebral blockade are recommended options for postoperative analgesia following posterolateral thoracotomy in adults (1) and these techniques are also useful and safe in children (2–4). Despite isolated case reports regarding the successful use of such advanced regional techniques in preterm infants, it appears in our opinion difficult to implement the widespread use of these modalities in extremely preterm infants. Thus, other effective regional anesthesia alternatives would be welcome for these patients when undergoing surgical ductus ligation.

An adult meta-analysis has found that the use of wound catheter techniques is surprisingly effective following thoracic and abdominal procedures (5) and successful use of wound catheter techniques has also recently been reported in children following sternotomy, iliac bone grafting, and minor–moderate abdominal surgery (6–8).

We have for several years used wound catheters as the primary analgesic technique following ductus ligation in extremely preterm infants and noted a more than 50% reduction in morphine consumption (9). However, during our development of this technique, we have experienced that intermittent bolus injections frequently are associated with wound leakage of the local anesthetic and that continuous infusions occasionally result in plasma levels close to what may cause systemic toxicity. Thus, an intermittent infusion strategy has emerged as the best current alternative.

The primary aim of the study was to determine the plasma levels of levobupivacaine (LB) associated with two alternative intermittent infusion techniques and thereby assess if they are associated with a reasonable margin of safety to toxic levels for long-acting local anesthetics. Secondary outcome parameters were pain scores, morphine consumption, need for rescue fentanyl administration, and wound healing without the intention of proving one strategy being superior to the other.

Methods

Following Ethics Committee (Dnr: 2008/482-31) approval and written parental informed consent, 18 preterm infants scheduled for surgical closure of a patent ductus arteriosus (PDA) were enrolled in the study (Table 1 patient population). The study has been performed according to the Declaration of Helsinki. Exclusion criteria were body weight <500 g at the time of surgery (due to limitation of total blood sampling volume), any previous adverse reaction to the administration of local anesthetics, lack of consent or participation in any other clinical research study.

General anesthesia protocol

All patients were mechanically ventilated with adequate arterial and venous lines in place. Before transfer from the incubator to the operating table, the infant received intravenous administration of atropine (10 $\mu g \cdot kg^{-1}$), fentanyl (10 $\mu g \cdot kg^{-1}$), and atracurium (0.5 $mg \cdot kg^{-1}$).

The patients were ventilated in pressure control mode (Dräger Primus; Dräger Company, Lübeck, Germany) and anesthesia maintained by sevoflurane 1.0–1.5% in oxygen/air. During surgery, hand ventilation was used intermittently as needed. An additional bolus of fentanyl (15 $\mu g \cdot kg^{-1}$) and atracurium (0.25 $mg \cdot kg^{-1}$) was given before the start of surgery. Additional fentanyl boluses were administered as needed (increase in heart rate or blood pressure by >20% compared to preincision), up to a maximum total dose of 50 $\mu g \cdot kg^{-1}$. A maintenance infusion of 2.5% glucose (Buffered Glucose 2.5%; B Braun, Melsungen, Germany) was given throughout the procedure at an infusion rate of 5–10 $ml \cdot kg^{-1} \cdot h^{-1}$. Intraoperative hypotension (reduction of >20% compared to preincision) was treated by bolus injections of 5–10 $ml \cdot kg^{-1}$ of albumin 5%.

Table 1 Patient population

Group	BII	DII
Sex (M/F)	6/3	4/5
Gest age at birth (weeks + days)	24 + 6 (24 + 0–26 + 4)	26 + 0 (24 + 3–29 + 3)
Age at op (weeks + days)	28 + 3 (26 + 0–32 + 0)	29 + 2 (26 + 6–33 + 2)
Weight at birth (g)	700 (564–937)	752 (600–1189)
Weight at op (g)	887 (642–1185)	840 (668–1650)

Wound catheter placement

Following closure of the muscle layers, a specially designed wound catheter (19G Pain Buster®, length 2.5 cm; I-Flow corporation, Lake Forest, CA, USA) was placed under direct vision by the surgeon before final closure of the wound. An introducer needle with sheath was fed subcutaneously from the medial angle of the wound to a point approximately 2–3 cm away (from the medial edge of the incision), and the catheter was inserted and placed along the length of the wound. The wound was then closed with continuous subcutaneous sutures and a continuous intracutaneous suture. Finally, the wound was covered with a transparent dressing (Figure 1). The wound catheters were scheduled to be removed 72 h postoperatively.

Bolus plus early start of an intermittent infusion (BII)

Following the application of the transparent dressing and after prefilling the catheter (0.08 ml), a manual bolus injection of LB was performed by the surgeon (0.625 mg·ml^{-1}, 0.2 mg·kg^{-1} = 0.32 ml·kg^{-1}). As soon as the infant had been transferred back to a normal and stable Neonatal intensive care unit (NICU) situation, a continuous infusion through the wound catheter was started. The infusion was switched every 8 h between LB and saline (for blinding purposes, please see Study design, statistics, and randomization below), starting with an 8-h infusion period of LB 0.625 mg·ml^{-1}, 2.0 mg·kg^{-1}·24 h^{-1} = 3.2 ml·kg^{-1}·24 h^{-1}.

This regimen was chosen based on the notion that immediate bolus administration of LB followed by active infusion for 8 h may result in optimization of the effect of this treatment modality.

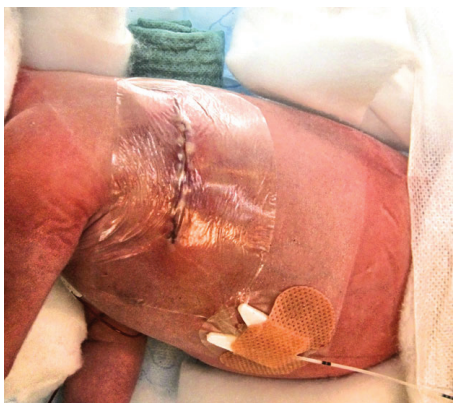

Figure 1 External catheter fixation and dressing.

Delayed start of intermittent infusion (DII)

In this group, the bolus injection was performed with saline, later followed by continuous infusion in the NICU in a similar fashion as for group BII. However, in the DII group, the infants first received an 8-h period of saline infusion. Thus, LB infusion was first started approximately 8 h after the end of the operation in this patient group.

This infusion strategy was based on the notion that the infants would have adequate postoperative analgesia from the intraoperative fentanyl load (25–50 μg·kg^{-1}) for the first 8–10 postoperative hours because fentanyl has a prolonged half-life in preterm infants (10). Thus, to provide additional analgesia by local anesthetics during this time period may be redundant and might potentially only result in unnecessary high plasma levels of LB.

Blood sampling

Blood samples were collected at 10, 13, 16, 19, 22, and 24 h after the first bolus (LB or saline) was given in the operating room. The dead-space of the tubings and the connectors were adequately cleared before the LB samples were taken. All samples were arterial except in one patient where the samples were taken from a central venous line. The maximum volume of blood collected at each time was 0.8 ml. All samples were immediately centrifuged for 10 min at 4042 ***g***. The plasma was then carefully pipetted and transferred to a preservative-free plastic tube and frozen at −18°C until analysis.

Levobupivacaine analysis

Quantification of total plasma LB was performed at the Department of Clinical Pharmacology, Karolinska University Hospital, Stockholm, Sweden, according to Good Laboratory Practice (GLP). The detection method is based on sample preparation by a two-step liquid–liquid extraction followed by separation and detection by liquid chromatography–mass spectrometry (LC-MS) using doxepin as internal standard. Quantification range of the method was 0.014–2.900 μg·ml^{-1}.

Method uncertainty was <10% (Coefficient of Variation).

Morphine infusions

Established clinical practice at our hospital has previously been to start a postoperative morphine infusion

when the patient returns back to the NICU. The standard infusion rate is set to 15 $\mu g \cdot kg^{-1} \cdot h^{-1}$ to prevent postoperative pain and to allow comfortable NICU care, including mechanical ventilation. However, for study purposes, we decided to instead start the postoperative morphine infusion at a reduced rate (10 $\mu g \cdot kg^{-1} \cdot h^{-1}$) to allow crude evaluation of the analgesic efficacy of the two infusion regimens (see below).

Postoperative pain scoring

The Echelle Douleur Inconfort Noveau (EDIN) pain score instrument is a validated pain score for use in preterm infants (11) and represents standard of care in our NICU. Assessment of pain was performed hourly and additional assessments were performed as needed in between the hourly recordings.

A pain score of 3–5 was set as the target range (min score: 0; max score: 15). A score below the target zone for six consecutive hours resulted in a reduction of the morphine infusion rate by 2.5 $\mu g \cdot kg^{-1} \cdot h^{-1.}$

A pain score above the target range resulted in administration of rescue analgesia (see below). If not corrected by a single dose of rescue analgesia, the morphine infusion rate was increased by 2.5 $\mu g \cdot kg^{-1} \cdot h^{-1}$.

Rescue analgesia

In case of pain score >5, the infant received an intravenous dose of fentanyl (1 $\mu g \cdot kg^{-1}$).

Signs of systemic local anesthetic toxicity

The nursing staff was specifically instructed to note any signs of ventricular cardiac arrhythmia or focal/generalized seizures.

Wound healing

The wound was inspected daily, and signs of leakage (presence of visible clear liquid under the transparent dressing) and infection were noted. Normal wound healing was defined as a scar free from signs of infection or inflammation at 10 days postoperatively.

Study design, statistics, and randomization

Study design

Based on our previous data showing a substantial and clinically relevant morphine sparing effect of the wound catheter technique (>50% reduction of postoperative morphine administration) (9), it was not considered ethical to include a placebo group to the study design. For rationale regarding the chosen intermittent infusion regimens, please see Discussion section.

Power calculation

As the primary aim of the present study was to determine the 24-h plasma concentration profile of LB associated with two different intermittent infusion regimens, no power calculation was performed with regard to the primary aim of the study.

Statistical methods

Mann–Whitney *U*-test was used when calculating differences between the two groups in terms of plasma concentrations. Classified data from two independent populations were compared by Fisher′s exact test.

Randomization procedure

The patients were randomized into two groups, BII and DII. The randomization was performed in blocks of 10 patients. The randomization codes were derived from computer-generated numbers and allocated using the sealed opaque envelopes technique.

Blinding procedures

To allow proper blinding, the study randomization was performed by the hospital pharmacy. The pharmacy provided syringes labeled with the patient number and in which order they were to be given with every second syringe containing either LB or normal saline. Every 8 h, the syringes were then switched according to the study protocol.

Results

In total, 18 preterm infants were enrolled and randomized: Group BII: $n = 9$ and group DII: $n = 9$. Two patients were excluded from the postoperative analgesia analysis: One patient from group DII was excluded due to an intraoperative surgical rift of the aorta, subsequently resulting in an unusual and complicated postoperative course. One patient in the BII group was excluded from the analgesia analysis due to a protocol violation with regard to the initial postoperative morphine infusion rate (Figure 3).

Levobupivacaine plasma concentrations

The total amount of LB administered during the first 24 h postoperatively was 1.963 $mg \cdot kg^{-1}$ in Group BII and 0.664 $mg \cdot kg^{-1}$ in Group DII.

As expected, the two infusion regimens were associated with different plasma concentration profiles, with

LB plasma levels being clearly higher in the BII group during the 10–16 postoperative period as compared to the DII group (Figure 2). However, during the latter part of the observation period (19–24 h), the plasma levels of LB between the two groups appeared to converge.

The maximal plasma concentration of LB in the BII group was 1.682 $\mu g \cdot ml^{-1}$ as opposed to 0.549 $\mu g \cdot ml^{-1}$ in the DII group. Median plasma concentrations of LB at 24 h were 0.342 $\mu g \cdot ml^{-1}$ and 0.106 $\mu g \cdot ml^{-1}$ in group BII and DII, respectively ($P = 0.0653$).

Postoperative pain scores

The median hourly EDIN pain score during the 24-h observation period was 0 (range: 0–15) in Group BII and 1 (range: 0–11) in Group DII (P = ns).

Need for rescue analgesia

Number of patients in need for rescue analgesia was lower in the BII group ($n = 2$) compared to group DII ($n = 7$) ($P = 0.0406$). The vast majority of the fentanyl doses ($n = 12$) in group DII was given during the 8–16-h postoperative period as opposed to only four doses during the 0–8 h period.

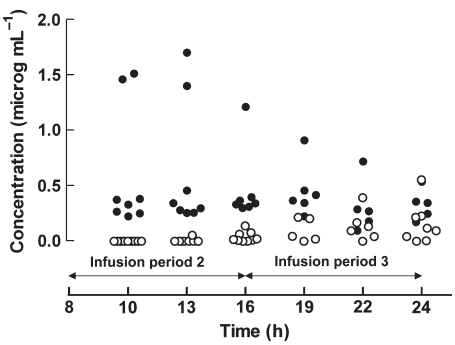

Figure 2 Plasma concentrations of levobupicacaine during infusion periods 2 and 3. ●, Concentrations in Group BII; ○, Concentrations in Group DII. During the infusion period 1 (0–8 h), patients in Group BII were treated with levobupicacaine and patients in Group DII were treated with saline. No sampling data were obtained during this period. During the infusion period 2 (8–16 h), patients in Group BII were treated with saline and patients in Group DII were treated with levobupicacaine. Sampling times: 10, 13, and 16 h. During the infusion period 3 (16–24 h), patients in Group BII were treated with levobupicacaine and patients in Group DII were treated with saline. Sampling times: 19, 22, and 24 h.

Total morphine consumption

The median morphine consumption was 272 $\mu g \cdot kg^{-1}$ (range: 170–494 $\mu g \cdot kg^{-1}$)·24 h^{-1} in group BII and 278 $\mu g \cdot kg^{-1}$ (range: 240–360 $\mu g \cdot kg^{-1}$)·24 h^{-1} in group DII (P = ns).

Changes in morphine infusion rate

In the BII group, six patients got their morphine infusion rate reduced during the study period (0–24 h) as opposed to only one patient in the DII group.

We could not see any clear connection between the reduction in morphine infusion rate and LB administration though.

Signs of systemic local anesthetic toxicity

No signs or symptoms of systemic local anesthetic toxicity were noted during the study.

Removal of the catheter

No signs of pain or discomfort were noted during the removal procedure.

Wound healing

In eight patients, a minimal amount of leakage fluid was detected under the transparent dressing. Signs of wound infection and a positive culture for staphylococcus and enterococcus were present in one patient on postoperative day 15 (group BII; treated by regular wound care and appropriate antibiotics). In all other patients ($n = 17$), the wound had healed as expected at 10 days postoperatively.

Discussion

The main finding of the present study was that both studied intermittent infusion regimens of local anesthetic through a wound catheter were associated with acceptable plasma levels of levobupivacaine as well as producing relevant postoperative analgesia in preterm infants undergoing surgical PDA repair.

Before discussing the study results, we would like to draw the reader's attention to some aspects associated with our study design. First, from a toxicity point of view, the assessment of the free plasma concentration of LB may be more closely linked to systemic toxicity than the total plasma concentration (12). However, the performance of proper free fraction analysis requires a considerable sampling volume for each individual sample (at least 3 ml of blood per sample), resulting in a

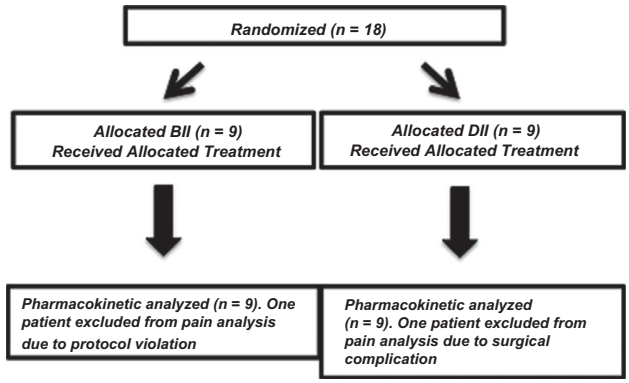

Figure 3 Consort diagram.

clearly excessive total blood sampling volume per patient. Therefore, to comply with the requirements of the Ethics Committee, we decided to analyze only the total plasma LB levels associated with the different infusion regimens. Second, the choice of design of the two administration regimens may need further clarification. When starting with this new treatment, we used an intermittent bolus injection technique (9). Although effective, this regimen was often associated with leakage through the wound itself or caused backflow alongside the catheter with substantial leakage occurring from the catheter insertion site. Against this background, continuous infusion appeared the better option.

However, our pilot data from the use of continuous infusion of LB were found to result in unwanted plasma levels of LB after 24 h of continuous infusion (regularly >1 $\mu g \cdot ml^{-1}$). As a pragmatic compromise, we decided to investigate two different administration protocols based on intermittent infusion of LB. Third, the limited number of patients studied in each group admittedly does limit the possibility to assess the secondary pharmacodynamic end points.

Plasma concentrations of levobupivacaine

Albeit both infusion regimens being associated with total plasma levels of LB that were below the proposed toxic plasma concentration threshold for racemic bupivacaine (2 $\mu g \cdot ml^{-1}$) (12), the BII protocol, as expected, resulted in higher plasma levels of LB compared to the DII regimen (Figure 2). Furthermore, the BII regimen was also associated with the presence of an outlier-individual that peaked at a plasma concentration (1.6 $\mu g \cdot ml^{-1}$) not too far from the potentially toxic threshold. In this context, one should also remember that the toxic plasma level in preterm infants may be considerably lower than the 2 $\mu g \cdot ml^{-1}$ threshold that is based on data from adults. One important circumstance of this aspect is that the level of alpha-1 acid-glycoprotein (AAGP), the protein that is mainly responsible for the pronounced protein-binding of local anesthetics, most likely is considerable lower in preterm infants than in adults. As an illustration of this issue, it has been shown that the levels of AAGP in term neonates are reduced by approximately 50% compared to adult values (13). Very few data regarding AAGP levels are currently available for preterm infants but one might speculate that the AAGP levels could be even lower due to the immaturity of the liver that is responsible for AAGP synthesis. This may in turn result in higher free plasma levels of LB than one normally would expect in adults and hence the potentially toxic threshold for total plasma levels may, thus, be substantially lower in preterm infants than in older subjects.

The choice of sampling times can of course be discussed. We decided, due to great limitations in terms of amount of blood that could be obtained, that the chosen time points were adequate for information of plasma concentrations from a safety stand point.

Postoperative analgesia

The finding of low average EDIN pain assessment values during the postoperative observation period in both treatment groups provides supportive evidence for the potential of this multimodal postoperative analgesia regimen, which combine a wound catheter infusion of local anesthetics with a low–moderate postoperative intravenous infusion of morphine. How-

ever, the fact that the pain assessments on average were lower than the target range in both groups does suggest that even lower morphine infusion rates could be used successfully.

Despite the observation of very similar pain assessment values as well as almost identical median morphine consumption during the first 24 postoperative hours in both groups, a significantly higher number of patients in Group DII did receive rescue fentanyl administration as compared to patients in Group BII. This could obviously be interpreted as an advantage for the BII regimen. However, it should be noted that the need for rescue fentanyl in the DII group was clustered to the time period associated with the start of the LB infusion in this patient group, thereby potentially indicating insufficient onset of the analgesic effect of the local anesthetic infusion during this time window in Group DII. If taken together the potential risk for occasional outlier-patients when using the BII concept (Figure 2), our clinical conclusion based on this study has been to routinely use the DII model but to start the intermittent infusion at 4 h after surgery instead of 8 h postoperatively.

A point of discussion when using wound infusions of local anesthetics is of course the proper mechanism of analgesia. Based on an extensive adult literature, it appears reasonable to expect that a local effect is responsible for the majority of the analgesic effect associated with this technique. However, because there is published evidence that intravenous infusions of local anesthetics can produce clinically relevant analgesia (14–16), it cannot be ruled out that systemic absorption of the local anesthetic may in part contribute to the overall analgesic effect resulting from the use of this novel therapeutic approach.

Wound healing

A special concern associated with the use of a wound catheter technique is whether it may negatively affect the wound healing process. We find it reassuring that all wounds healed as expected except one patient experiencing a culture-verified superficial wound infection.

In conclusion, the use of both studied intermittent infusion regimens in preterm infants undergoing surgical ductus ligation was found to result in plasma levels of levobupivacaine below what may be considered associated with a risk for systemic toxicity. Furthermore, both regimens were associated with low postoperative pain scores.

Funding

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Conflict of interest

P.A.L. is a member of the Editorial Board of British Journal of Anaesthesia and a section editor of Pediatric Anaesthesia. The authors report no conflict of interest.

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Eur J Anaesthesiol 2015; **32:**851–856

ORIGINAL ARTICLE

Plasma levels of levobupivacaine during continuous infusion via a wound catheter after major surgery in newborn infants

An observational study

Joachim Krylborn, Marie E. Anell-Olofsson, Catarina Bitkover, Stefan Lundeberg, Marco Bartocci, Carl-Olav Stiller and Bjorn A. Larsson

BACKGROUND Epidurals may be challenging in neonatal patients due to technical difficulties relating to insertion and the risk of local anaesthesia toxicity. The use of wound catheters with an infusion of local anaesthetic has been shown to be well tolerated in adults and older children. There are few data concerning wound catheter techniques in neonatal patients.

OBJECTIVES The primary aim of this study was to analyse plasma levels of levobupivacaine associated with continuous wound infiltration via a catheter following neonatal surgical procedures. Secondary parameters, including the quality of postoperative analgesia and wound healing, were also noted.

DESIGN A prospective, observational study.

SETTING Paediatric ICU at the Karolinska University Hospital, Stockholm, Sweden, from March 2008 to December 2010.

PATIENTS Twenty newborn infants (median weight 3.48 kg) scheduled for major abdominal or thoracic surgery were included. Exclusion criteria were known or suspected hepatic dysfunction. Before skin closure, a subcutaneous catheter was inserted into the wound followed by a 0.5 mg kg^{-1} bolus of levobupivacaine (0.125%, 0.4 ml kg^{-1}) through the catheter. A continuous infusion was started 20 to 30 min later at a rate of 0.2 mg kg^{-1} h^{-1} (0.16 ml kg^{-1} h^{-1}).

MAIN OUTCOME MEASURES Plasma concentrations of levobupivacaine (total and unbound) at 12, 24, 48 and 72 h postoperatively. Morphine consumption, pain scores and wound healing were also analysed.

RESULTS Median concentrations of unbound and total levobupivacaine at 72 h were 0.018 and 1.305 μg ml^{-1}, respectively. In 18 out of 20 infants [90%; 95% confidence interval (CI) 68.3 to 98.8], the unbound plasma concentration of levobupivacaine remained relatively stable and below 0.05 μg ml^{-1} throughout the 72 h observation period. Pain scores and morphine consumption levels were low. All wounds except one healed within 10 days.

CONCLUSION The studied infusion regimen was associated with plasma levels of levobupivacaine well below those associated with toxicity. Adequate wound healing, low pain scores and a reduced need for opioids were also noted.

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Introduction

In adult patients, the administration of continuous infusions or intermittent boluses of local anaesthetics through catheters placed into the surgical wound has become increasingly popular due to its simplicity, efficacy and safety.[1,2] Positive paediatric pilot studies using continuous wound catheter techniques have recently been published with regard to their use in association with cardiac surgery, iliac bone graft harvesting, orthopaedic and abdominal surgery.[3–5] However, to our knowledge, the use of wound catheters in neonates has not been described.

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During the last few years, we have accumulated experience in the continuous administration of levobupivacaine via wound catheters for postoperative analgesia following various types of neonatal surgical procedures. There is a need for data regarding the plasma levels of local anaesthetics associated with the use of such wound catheters in order to safeguard against the risk of provoking local anaesthetic systemic toxicity.

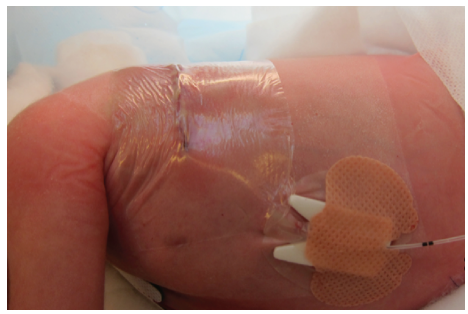
The primary aim of this study was to analyse the plasma levels of levobupivacaine associated with continuous wound infiltration following neonatal surgical procedures. Secondary outcome parameters, including the quality of postoperative analgesia, wound healing and time to enteral feeding and endotracheal extubation were also noted.

Materials and methods

Ethical approval for this study (Dnr: 2007/1462-31) was provided by the ethical committee of Karolinska Institutet, Stockholm, Sweden (Chair Pierre Lafolie) on 10 March 2008 and the study was performed in accordance with the declaration of Helsinki. After written parental informed consent, 20 newborn infants (<1 month of age) scheduled for major abdominal or thoracic surgery were included in the study. Exclusion criteria were known or suspected hepatic dysfunction.

A standardised anaesthetic technique was used. Premedication was not used in any of the patients. Anaesthesia was induced by atropine (10 to 20 $\mu g\,kg^{-1}$), fentanyl (1 to 2 $\mu g\,kg^{-1}$) and thiopentone (6 to 8 $mg\,kg^{-1}$) or propofol (2 to 3 $mg\,kg^{-1}$). Endotracheal intubation was facilitated by succinylcholine (2 to 3 $mg\,kg^{-1}$). Anaesthesia was then maintained by sevoflurane (2 to 4%) in an oxygen/air mixture. Additional fentanyl boluses (1 to 2 $\mu g\,kg^{-1}$) were administered at the discretion of the attending anaesthetist. At the request of the surgeon, further muscle relaxation was provided if required by the administration of a bolus of atracurium (0.5 $mg\,kg^{-1}$). Following closure of the muscle layers, the surgeon placed a multiorifice wound 19-gauge catheter (ON-Q PainBuster; BBraun Melsungen AG, Melsungen, Germany) of length 2.5 or 6.5 cm according to the length of the surgical incision, superficial to the muscle fascia under direct vision. The subcutaneous tissue was subsequently closed by single sutures and the skin was sutured by an intracutaneous suturing technique. A transparent dressing was also applied (Fig. 1). Immediately after skin closure, a bolus dose of 0.5 $mg\,kg^{-1}$ levobupivacaine (0.125%, 0.4 $ml\,kg^{-1}$) was administered through the wound catheter. After surgery, all children were transferred to the paediatric ICU (PICU) for postoperative care. On admission to the PICU, 20 to 30 min after the initial bolus, a continuous infusion of levobupivacaine 0.125% was started at a rate of 0.2 $mg\,kg^{-1}\,h^{-1}$ (0.16 $ml\,kg^{-1}\,h^{-1}$) using an infusion pump (Hospira GemStar; Hospira Inc., Lake Forest, Illinois, USA). The intended duration of the postoperative levobupivacaine infusion was 72 h. The catheters were removed after 72 h simply by gently pulling them out after removal of the dressing.

Fig. 1



Wound catheter with external dressing applied following thoracotomy. (Reproduced with permission from the parents).

The nurses were specifically instructed to monitor for signs of local anaesthetic toxicity, namely any signs of cardiac arrhythmia or local or generalised seizures. Examination by a paediatric anaesthetist was called upon if needed. Data from the monitoring system were also obtained for analysis.

All patients were treated according to an established multimodal sedoanalgesic PICU protocol, which included the supplementation of regional anaesthesia by the use of intravenous (i.v.) paracetamol (15 to 20 $mg\,kg^{-1}$ three to four doses per 24 h) and clonidine (1 $\mu g\,kg^{-1}$ three to four doses per 24 h or as a continuous infusion 5 to 8 $\mu g\,kg^{-1}\,24\,h^{-1}$). Opioids were given if indicated, for example, due to high pain scores. Pain was recorded every 6 h during the 72 h postoperative observation period using the validated Astrid Lindgrens Children's Hospital Pain Scale (ALPS) (0, no pain at all to 10, worst pain imaginable).[6] A pain score less than 3 is considered acceptable, a score of 3 to 5 indicates the need for intervention, often in form of nursing care, and a score more than 5 almost always indicates the need for treatment with analgesics. All patients were assessed and treated individually according to pain and comfort. The amount of postoperative opioid administration during the observation period was retrieved from the PICU patient data management system (CliniSoft database; CliniSoft Corporation, Wesley Chapel, Florida, USA).

The wound was inspected at least once daily until postoperative day 10 (perceived as the normal time for wound healing to occur). The following items were specifically noted: leakage; presence of erythema around the wound; signs of infection; and presence of normal wound healing at postoperative day 10.

Blood samples (1.5 ml each) were taken from indwelling venous or arterial catheters (depending on availability) at 12, 24, 48 and 72 h postoperatively. The blood samples were subsequently centrifuged (Centrifuge 5804R; Eppendorf AG, Hamburg, Germany) at 3000 rpm for 10 min. The plasma was then separated and stored at −20°C until final analysis. Separate blood samples for the determination of alpha-1-acid glycoprotein (AAG) were taken at induction of anaesthesia and again at 72 h postoperatively. Levobupivacaine analyses were performed at the Department of Clinical Pharmacology at the Karolinska University Hospital according to Good Laboratory Practice (GULP). Alpha-1-acid glycoprotein was quantified at the Clinical Chemistry Laboratory at Karolinska University Hospital Solana. Total levobupivacaine concentration was measured with reversed phase high-pressure liquid chromatography (HPLC) with mass elective detection (API-ES positive ion), using the Agilent 1100 series system (Agilent Technologies, Waldbronn, Germany). The range for quantification was 1.2 to 10 000 nmol l^{-1} and the limit of detection was 0.35 nmol l^{-1}. The method has a measurement insecurity of less than 5% and was calibrated for every analysis. Separation of the plasma proteins was accomplished by the use of a regular ultrafiltration system (Amicon centrifuge, Bedford, Massachusetts, USA). The unbound concentration of levobupivacaine was subsequently analysed using the same method as described above. Alpha-1-acid glycoprotein was analysed by rate nephelometry (IMMAGE; Beckman Coulter Inc., Brea, California, USA). The range for quantification was 0.35 to 108 g l^{-1}.

Enteral feeding was attempted if signs of bowel movements were present (as determined by auscultation, flatulence or defaecation) or if gastric aspirates were visibly clear and of minimal volume. Enteral feeding was deemed successful if it did not result in gastric retention or vomiting. The time to establishment of successful enteral feeding was noted.

As this was a purely observational study, no power calculation was performed. On the basis of prior plasma concentration studies,[7] we aimed to recruit 20 neonates to this study. Plasma concentration data are expressed as median (range). Pain scores are presented as median values. The 95% confidence intervals (CIs) were calculated as stated by Ott and Mendenhall.[8]

Results

Population demographics and the surgical diagnoses are shown in (Tables 1 and 2) respectively. Individual patient data for total plasma levobupivacaine concentrations are shown in Fig. 2a. Total postoperative levobupivacaine plasma concentrations were 0.80 (0.17 to 1.92), 1.52 (0.06 to 2.05), 1.37 (0.14 to 3.15) and 1.30 (0.08 to 2.68) μg ml^{-1} at 12, 24, 48 and 72 h, respectively. In most infants, the total concentration of levobupivacaine was found to increase during the first 48 h, followed by a slight decrease from 48 to 72 h. The maximum total levobupivacaine plasma concentration noted in the study was 3.15 μg ml^{-1}.

Table 1 Patient demographics

Sex (male/female)	14/6
Gestational age at birth (weeks + days)	39 + 6 (36 + 4 to 41 + 5)
Postnatal age at time of surgery (days)	4 (1 to 30)
Weight (kg)	3.48 (2.87 to 4.29)

Data are median (range) or number.

Individual patient data for unbound plasma levobupivacaine concentrations are shown in Fig. 2b. Unbound postoperative levobupivacaine plasma concentrations were 0.014 (0.002 to 0.05), 0.026 (0 to 0.059), 0.027 (0 to 0.208) and 0.017 (0 to 0.084) μg ml^{-1} at 12, 24, 48 and 72 h, respectively. In 18 out of 20 infants (90%; 95% CI 68.3 to 98.8), the unbound plasma concentration of levobupivacaine remained relatively stable and less than 0.050 μg ml^{-1} over the entire 72 h observation period. The maximum unbound levobupivacaine plasma concentration noted in the study was 0.210 μg ml^{-1} at 48 h.

Individual patient data for AAG are shown in Fig. 3. Alpha-1-acid glycoprotein levels increased in all but four patients. The unbound fraction of levobupivacaine at 72 h was 1.48 (0.4 to 4.8)%. No correlation between AAG at 72 h and the unbound fraction of levobupivacaine was observed. No signs or symptoms of local anaesthetic systemic toxicity were observed during the study period.

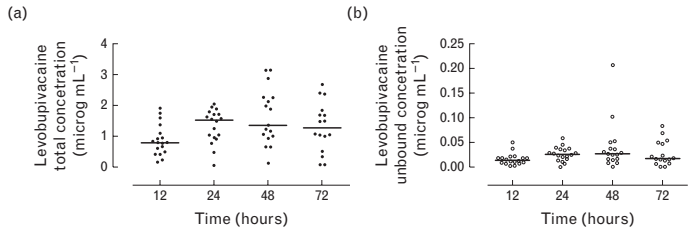
The ALPS scores were found to be low throughout the entire 72 h observation period at 2, 1, 0 and 1 at 12, 24, 48 and 72 h, respectively. In three patients, no supplemental opioid was administrated during the observation period. In the remaining 17 patients, the total amount of opioid given during the first 72 h ranged from 0 to 1.40 mg kg^{-1}.

In seven infants out of 20 (35%; 95% CI 15.4 to 59.3), some limited erythema around the wound could be observed during the infusion of levobupivacaine. Leakage either at the edges of the wound or at the catheter insertion site was noted in five patients; in one of these patients, the infusion was stopped slightly prematurely (at 68 h) due to excessive leakage. In this patient, the 72 h blood samples were obtained at 68 h. In 18 patients out of 20 (90%; 95% CI 68.3 to 98.8), the wound had healed in the expected manner by postoperative day 10. One

Table 2 Surgical diagnosis

Diagnosis	Number
Colonic atresia	1
Congenital diaphragmatic hernia	5
Pulmonary cyst	3
Duodenal atresia	3
Duodenal stenosis	1
Malrotation of the intestine	1
Oesophageal atresia	5
Tracheoesophageal fistula	1

Fig. 2



(a) Total serum concentrations of levobupivacaine during the study period. Median values are represented by the horizontal bar. (b) Unbound serum concentrations of levobupivacaine during the study period. Median values are represented by the horizontal bar.

patient had a local reaction to a suture protruding through the wound and one patient had a verified wound infection with *Staphylococcus aureus*. The infected wound had healed by postoperative day 10 with adequate treatment.

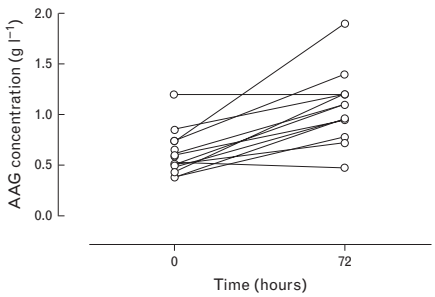
In four patients out of 20 (20%; 95% CI 5.7 to 43.7), enteral feeding could be started within 24 h. Within 48 h, 14 infants out of 20 (70%; 95% CI 45.8 to 88.1) were able to tolerate enteral feeding. Enteral feeding had successfully been restarted postoperatively in all patients within 96 h.

Within 24 h, 12 infants out of 20 (60%; 95% CI 36.0 to 80.9) had undergone successful tracheal extubation. The cumulative tracheal extubation rate was 75% by 48 h and 95% by postoperative day 5. One patient suffered from pulmonary hypertension and stayed on mechanical ventilation, including the use of inhaled nitric oxide, for an extended period of time.

Discussion

The main findings of the present study were that continuous wound catheter infusion of levobupivacaine according to our regimen resulted in plasma concentration levels well below proposed toxic thresholds and that no signs or symptoms of local anaesthetic systemic toxicity were noted. Secondary outcomes (postoperative pain scores, wound healing and time to enteral feeding and endotracheal extubation) also showed favourable outcomes.

Fig. 3



Alpha-acid-glycoprotein levels preoperatively and 72 h postoperatively.

Regional analgesia has become increasingly popular in paediatric patients due to the excellent pain relief that it provides. Recent studies have also indicated that the use of epidural analgesia appears to be associated with acceptable safety even in infants.[9] However, case reports referring to catastrophic outcomes following the attempted use of epidural blockade in neonates have been published[10,11] and the safety of using the guidelines for infusion rates of local anaesthetics in neonates for longer than 48 h are being questioned.[7] Thus, despite the merits of epidural analgesia, there is a need for further improvement, especially with regard to safety issues.[12] Before commenting further on our results, we would like to point out that, in our opinion and based on the wealth of published data and longstanding track record, continuous epidural analgesia remains the gold standard for postoperative analgesia in neonates subjected to major surgical interventions. Thus, further prospective, comparative randomised controlled trials are needed before the place of continuous wound catheter techniques in paediatric clinical care can be established.

The concept of a continuous infusion of local anaesthetic into the surgical wound area relies on two separate mechanisms of spread of the local anaesthetic agent to eventually reach the peripheral nerve endings and small peripheral nerve branches. First, vertical bulk transfer is anticipated to take place between the various layers of the surgical incision, thereby producing anaesthesia of both the skin and subcutaneous tissue as well as the underlying muscle layers. Second, local horizontal diffusion within each tissue layer is assumed to result in a varying degree of radial distribution to the area immediately adjacent to the incision. These two mechanisms are dependent on both the concentration of local anaesthetic agent and the volume per hour that is infused through the catheter.

Considering that the surgical wound in adults on occasions can involve both a considerable incisional length and a substantial wound depth, it is slightly surprising that wound catheter techniques can be successfully employed (e.g. Pfannenstiel incisions in gynaecological and obstetric surgery).[13] However, a number of reviews and meta-analyses do show that wound catheter techniques represent a useful therapeutic option for postoperative analgesia in adults.[14,15] In contrast, the size of the surgical incision is substantially smaller in children, which is especially true in the neonatal situation wherein the incision length and tissue depth is minimal. Furthermore, the texture of the tissues in neonates is generally more favourable for diffusion than adults. Thus, neonates should on theoretical grounds provide almost ideal conditions for wound catheter analgesia to be efficacious.

On the basis of the difficulties and risks associated with the insertion of epidural catheters in very low-weight babies (<1500 g) for a number of years, we have used a wound catheter technique in preterm infants undergoing surgical closure of patent ductus arteriosus.[16] On the basis of a greater than 50% reduction in postoperative morphine administration in infants treated with wound catheters as compared with no regional anaesthesia (unpublished data), we decided to perform the current study in neonates as a pilot for further prospective, comparative, randomised clinical trials. A special focus of the present study was to identify a well tolerated and effective local anaesthetic infusion regimen in this population that could be used in future clinical trials.

The dose and concentration of levobupivacaine used in our study was based on our previous clinical experience over several years. The dose and volume is also the same as we have used in lumbar epidurals in similar patients.[7]

No specific data with regard to the plasma levels associated with early or manifestations of local anaesthetic toxicity are currently available for levobupivacaine. However, it is reasonable to expect that such values should be somewhat higher than those for racemic bupivacaine. Thus, if compared with the potentially toxic plasma concentrations of racemic bupivacaine (unbound bupivacaine 0.3 $\mu g\,ml^{-1}$, total bupivacaine 4 $\mu g\,ml^{-1}$),[17,18] all our measured levobupivacaine concentrations fell within what is accepted as a reasonable safety margin. In eight patients, we noted a tendency towards higher total concentrations of levobupivacaine. These patients showed no differences in terms of operation mode, postnatal age or preoperative medical status compared with the rest of the study population. Despite slowly rising total plasma concentrations during the time period of 0 to 48 h (Fig. 2a), the unbound concentration that is linked to potential toxicity remained remarkably stable in the vast majority of babies over the 72 h postoperative observation period (Fig. 2b). As AAG generally has a high affinity for local anaesthetics, the observation of stable unbound concentrations of levobupivacaine can most likely be explained by the increase in postoperative AAG levels (Fig. 3), which represents an integral part of the acute phase reaction that is triggered by surgery and the associated neuroendocrine surgical stress response.[19] The patient who had the highest unbound concentration of levobupivacaine at 48 h also had a high total concentration at the same time. There was no correlation to low AAG levels at 72 h. This patient underwent thoracotomy for repair of oesophageal atresia and had no signs of hepatic or renal impairment before or after surgery. The stable concentrations of unbound levobupivacaine seen during the 72 h postoperative observational period, together with the absence of any signs indicative of local anaesthetic toxicity, appear to support the clinical safety of the infusion regimen used in this study.

The combined use of our wound catheter regimen with a standardised analgesic protocol was found to result in a favourable pain score profile during the 72 h postoperative observation period, indicating clinically satisfactory analgesia. The observation that three patients did not need any additional administration of opioids at all during the observation period points to the analgesic potential of the wound catheter technique. Compared with the standard continuous opioid (morphine) infusion rate (20 $\mu g\,kg^{-1}\,h^{-1}$) used in neonates not receiving regional anaesthesia in our centre, only one catheter appeared not to be associated with any clinically relevant opioid-sparing effect. The observations that the infants could undergo tracheal extubation and resume enteral feeding in line with what is normally seen with the use of epidural analgesia in this age group appear to support the postoperative efficacy of the wound catheter technique used.

For obvious reasons, various wound-related parameters are of special interest when wound catheter infusions are used. In 18 patients (90%), normal wound healing was observed. The observed reaction to a protruding suture is, in our opinion, not related to the wound catheter technique *per se* but instead due to the surgical suturing technique. Only one case of suboptimal wound healing (culture verified infection) was noted, which eventually healed without excessive scaring following local treatment and adequate antibiotic therapy. The erythema that could be observed around the wound during the infusion of local anaesthetic in 33% of the neonates is most likely due to local vasodilatation caused by levobupivacaine and the erythema subsided soon after discontinuation of the infusion in all cases. Excessive leakage of infused local anaesthetic occurred in one patient and the infusion was terminated slightly earlier than initially planned (68 vs. 72 h). On the basis of the experience gained from this limited cohort, wound catheter infusions of levobupivacaine do not appear to interfere with the wound healing process to any major extent.

This study has some limitations that need consideration. First, due to the special circumstances associated with the care of neonates, the blood samples were collected from the best available intravascular catheter, which resulted in blood samples being taken either from an arterial line or a venous catheter. It is well known that arterial and venous plasma concentrations differ in the immediate phase following a bolus injection of local anaesthetic as part of a single injection block technique.[20] However, as the first blood sample was taken 12 h after the start of the bolus along with continuous infusion regimen, it is reasonable to assume that arterial and venous blood samples should have equilibrated with regard to levobupivacaine concentration; this assumption is even more probable for the samples collected during the later phase of the observation period. Thus, we believe that this minor imperfection in study design, necessitated by clinical reality, will not have any major influence on the final study results. Second, although sufficient to allow a reasonable estimate of individual plasma concentration profiles associated with our neonatal infusion regimen, the sample size is too small to allow any far-reaching conclusions with regard to the secondary parameters investigated in the present study. Third, it is possible that higher plasma concentrations of levobupivacaine could have occurred in conjunction with the administration of the bolus dose. A specific issue when trying to interpret the postoperative pain assessments is that all children, apart from the standardised infusion of levobupivacaine, received varying amounts of multimodal sedoanalgesia that was required in order to permit mechanical ventilation and PICU care. Thus, the overall estimation of postoperative analgesia associated with our wound catheter regimen must be interpreted with a certain degree of caution. However, the combined picture of the secondary parameters is, in our opinion, indicative of our wound catheter regimen being associated with an acceptable degree of clinical efficacy.

In conclusion, total and unbound plasma concentrations of levobupivacaine remained within acceptable safety limits when using our wound catheter infusion regimen in neonates undergoing neonatal surgery. Furthermore, our regimen was associated with low pain scores, apparently normal postoperative recovery and was not associated with any adverse effects with regard to wound healing. The results of the study do in our opinion merit the performance of an adequately sized, prospective randomised trial comparing the wound catheter technique with continuous epidural analgesia in the context of postoperative analgesia after neonatal surgery.

Acknowledgements relating to this article

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Conflicts of interest: none.

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Clinical Investigation

CLINICAL INVESTIGATION

Plasma concentrations of alpha-1-acid glycoprotein in preterm and term newborns: influence of mode of delivery and implications for plasma protein binding of local anaesthetics

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Abstract

Background: Alpha-1-acid glycoprotein (AAGP) is an acute-phase protein with high affinity for amide local anaesthetics (LAs), and a major determinant of free and potentially toxic concentrations of LAs in plasma. Neonates are known to have lower plasma concentrations of AAGP than adults, and are at risk of developing high free concentrations of LAs. Data regarding AAGP in newborns are so far sparse. The aim of this study was to determine plasma concentrations of AAGP after delivery of preterm and term infants, and to investigate correlations between AAGP and gestational age, birth weight, gender, and mode of delivery.
Methods: In this prospective observational study, blood was sampled from umbilical cords of 70 newborn infants born at gestational weeks 27–42 immediately after delivery. Blood samples were subsequently analysed for AAGP plasma concentrations with an immunoturbidimetric assay.
Results: We found higher concentrations of AAGP in infants born vaginally compared with those who were delivered by elective Caesarean section [median (inter-quartile range) 0.189 g litre^{-1} (0.142–0.263 g litre^{-1}) *vs* 0.110 g litre^{-1} (0.094–0.157 g litre^{-1}; P=0.0003)], respectively. There was a correlation between gestational age and AAGP concentrations (r=0.50; P=0.011), with significantly higher concentrations in the more mature infants. Gender and birth weight did not appear to influence the plasma concentrations of AAGP.
Conclusions: Alpha-1-acid glycoprotein concentrations in newborns are influenced both by gestational age and mode of delivery. Thus, when dosing local anaesthetics in a parturient, these factors should be taken into account.

Keywords: anaesthesia; local; glycoproteins; infant; newborn

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Editor's key points

- The acute-phase-protein alpha-1-acid glycoprotein (AAGP) has a high affinity for amide local anaesthetics.
- Lower AAGP concentration is associated with reduced binding of amide local anaesthetics, and may increase the risk for local anaesthetic toxicity.
- Newborns have lower AAGP concentrations than adults, and AAGP concentrations increase after 260 days of gestation.
- AAGP concentrations can also increase during stressful situations, such as a normal vaginal delivery or Caesarean section, and could therefore influence the free concentration of local anaesthetics and the development of local anaesthetic toxicity.

The administration of local anaesthetics (LAs) by epidural or wound catheter infusion represents valid options to provide good postoperative analgesia after major surgery in both term and preterm newborn infants.[1–5] However, excessive dosage, unintentional intravascular administration, or rapid systemic absorption of LAs may cause serious complications, such as seizures and haemodynamic collapse.[6–8] Relevant safety issues need to be addressed before recommending the widespread use of regional anaesthesia in these patient categories.

LAs have a high affinity for plasma proteins making the free fraction of key concern regarding potential LA systemic toxicity (LAST). Alpha-1-acid glycoprotein (AAGP), an acute-phase protein, is mainly responsible for the plasma binding of LAs.[9] Low concentrations of AAGP are associated with reduced binding of LAs and result in higher free plasma concentrations of LAs that, in turn, will increase the risk for LAST. It has been shown that plasma concentrations of AAGP in newborns are approximately half of adult levels.[10] LAs also bind to albumin and red blood cells.[11] As albumin concentrations also are reduced in newborn and especially preterm infants, it will probably not provide any additional safety regarding LAST. The high haematocrit of newborn infants is only of limited importance.[12] To our knowledge, AAGP concentrations have not been determined in the very preterm population; furthermore, the effect on AAGP concentrations caused by mode of delivery (vaginal delivery *vs* Caesarean section) remains unexplored. This is useful knowledge to avoid LAST and make dosage suggestion in these patient categories.

The aim of this prospective observational study was to determine AAGP plasma concentrations in preterm and term newborns, and whether there was a correlation between AAGP concentrations and gestational age, birth weight, gender, and mode of delivery.

Methods

Study population

Ethical approval for this study (Dnr:2014/64-31/2) was provided by the ethical committee of Karolinska Institutet, Stockholm, Sweden on April 19, 2014. After written parental informed consent, 70 newborn infants were included in the study.

Blood sampling and measurements

Immediately after delivery, 1 ml blood was sampled from the umbilical artery attached to the placenta. The blood was collected in micro-cuvettes (Microvette® 500 K2-ethylenediaminetetraacetic acid (EDTA) 500 ml; Sarstedt AG and Co., Nümbrecht, Germany) and sent for further handling in the hospital laboratory. In the laboratory (Karolinska University Hospital, Huddinge, Sweden, accredited in accordance with the International Standard ISO 15189:2012), the micro-cuvettes were centrifuged at 20°C for 10 min using the Sigma 1–14K centrifuge (LABEX Instrument AB, Osterode am Harz, Germany). Plasma AAGP (orosomucoid) was analysed in EDTA plasma with an immunoturbidimetric assay, cobas C system (Roche Diagnostics, Mannheim, Germany). The lower limit of quantification was 0.1 g litre^{-1}, defined as three standard deviations above the lowest standard. The total coefficient of variation was 5%.

Investigated parameters

The AAGP concentrations were analysed in relation to gestational age, birth weight, gender, and mode of delivery (vaginal delivery *vs* Caesarean section).

Statistical analyses

Median values and inter-quartile range (IQR) were used when describing AAGP concentrations. Kruskal–Wallis test was used to compare concentrations of AAGP between infants born with different modes of delivery. To investigate the correlation between AAGP and gestational age, the Spearman correlation test was used. Statistics were evaluated by GraphPad InStat 3.10 (GraphPad Software, La Jolla, California, USA).

Results

The patient characteristics of the included newborns are presented in Table 1. Six infants were excluded because of

Table 1 Patient characteristics. AAGP, alpha-1-acid glycoprotein; IQR, inter-quartile range.

	Modes of delivery		
	Vaginal	Elective Caesarean section	Acute Caesarean section
Number of infants (*n*)z	31	26	13
Gender (male/female)	17/14	16/10	7/6
Gestational age at birth (days) (median: minimum–maximum)	273 (191–295)	265 (222–279)	263 (190–293)
Body weight (g) (median: minimum–maximum)	3363 (1734–4630)	3439 (1234–4790)	2873 (1058–4000)
AAGP concentration (g litre^{-1}) (median: IQR)	0.189 (0.142–0.263)	0.110 (0.094–0.157)	0.182 (0.121–0.376)

missing values from the laboratory. The median plasma concentration of AAGP in cord blood was 0.158 g litre^{-1} (IQR 0.110–0.216).

Plasma concentrations of AAGP in relation to gender or birth weight

The median plasma concentrations of AAGP were 0.148 g litre^{-1} (IQR 0.110–0.240) and 0.160 g litre^{-1} (IQR 0.102–0.210) in male and female newborns, respectively (P=0.988). We found no correlation between AAGP concentrations and birth weight (r=0.18; P=0.162).

Plasma concentrations of AAGP in relation to mode of delivery or gestational age

The median plasma concentrations of AAGP were 0.110 g litre^{-1} (IQR 0.094–0.157) and 0.189 g litre^{-1} (IQR 0.142–0.263) in newborn infants delivered by elective Caesarean section and vaginally, respectively (P=0.0003; Fig. 1). The median plasma concentration of AAGP was 0.182 g litre^{-1} (IQR 0.121–0.376) in the group delivered by acute Caesarean section. The concentration of AAGP remained stable until 260 gestational days. Thereafter, the AAGP concentrations augmented with increasing gestational age (r=0.50; P=0.011; Fig. 2). AAGP concentrations were as high as 0.78 and 0.42 g litre^{-1} in two infants born <220 days of gestation (delivered vaginally and by acute Caesarean section, respectively). The third infant born <220 days of gestation had an AAGP concentration of 0.14 and was delivered vaginally.

Discussion

The acute-phase protein AAGP has a high affinity for amide LAs and is a major determinant of free and potentially toxic concentrations of LAs in plasma. The main finding of our study was that AAGP plasma concentrations in newborn infants are low (approximately 20% of normal adult concentrations)[13] and appear stable at a low concentration during 260 days of gestation. AAGP concentrations were significantly higher in infants born by normal vaginal delivery compared with

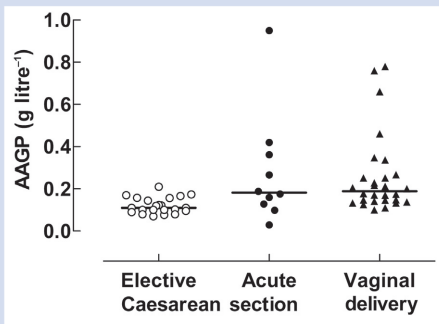

Fig 1. Median alpha-1-acid glycoprotein (AAGP) concentrations in newborn infants delivered by elective and acute Caesarean section or vaginally.

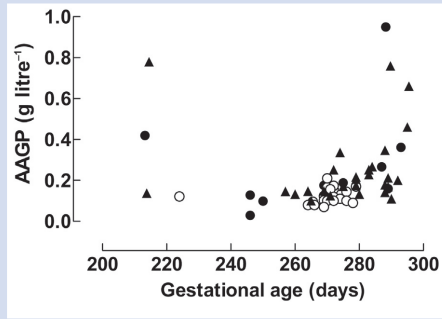

Fig 2. Alpha-1-acid glycoprotein (AAGP) concentrations during gestational age.

elective Caesarean section. Among the participants in our study, there were two infants born <220 gestational days who had high concentrations of AAGP presumably as a response to the stress of being born (one being born vaginally and the other by acute Caesarean section). This may indicate that even very preterm infants can increase their AAGP production in response to stress.

Preterm and term newborn infants are occasionally subjected to major surgery and need the best possible postoperative analgesia. Morphine and other opioids are frequently used, but their routine use has recently been debated.[14–16] The trend in adult postoperative care is moving away from routine use of opioids for various reasons.[17,18]

Regional anaesthetic techniques are now used in children[2] and can be used even in extremely preterm infants.[19] However, unintentional overdosing, inadvertent intravascular administration, or excessive absorption of LAs from the injection site may cause serious side-effects (e.g. seizures and haemodynamic collapse).[6–8]

In earlier studies, we have found acceptable plasma concentrations of LAs regarding the risk of LAST, combined with the lack of observed side-effects both in preterm and term newborn infants.[19] Even so, there are still unresolved safety issues especially in preterm infants. Defining the AAGP plasma concentrations also in the preterm population is of importance, as low concentrations will reduce plasma protein binding, thereby increasing the free and potentially toxic plasma concentration of LAs.

Plasma concentrations of AAGP in preterm and term newborns

Previous studies have shown that AAGP plasma concentrations in infants are lower than in adults, and that AAGP does not reach adult values until 1 yr of age.[10] Our results show that AAGP concentrations appear to remain stable at a low level until 260 days of gestational age when the levels start to rise significantly. One interpretation of this finding is that the hepatic production of AAGP is immature until about 260 days (36 gestational weeks) when the liver enzyme maturation results in increased synthesis of the glycoprotein.

Increased plasma concentrations of AAGP are part of the acute-phase-protein response that is triggered by various

modes of stress and constitutes one aspect of the innate immune response. It is well known that vaginal delivery is associated with a substantial endocrine stress response that is considerably higher than what is observed in association with elective Caesarean section.[20] It has hitherto been unknown whether the stress of vaginal delivery is substantial enough to trigger an increased production of AAGP. In line with our prediction, AAGP concentrations were significantly higher after normal vaginal and acute Caesarean delivery than after elective Caesarean section. The number of infants in the age range <260 days (27–36 gestational weeks) was too small to allow for a separate *post hoc* analysis, but a similar pattern was observed also in this patient category.

Study limitations

Despite the fact that all potential mothers gave consent during the inclusion period, the actual number of preterm infants (<37 gestational weeks) was numerically small (n=14). This is, of course, a limiting factor that precluded a proper subgroup analysis of these subjects concerning the effect by mode of delivery. To analyse the influence of the low levels of AAGP on the *in vitro* free fraction of LAs would, of course, have provided further data of interest. To determine plasma protein binding properly would need a total plasma volume of approximately 5 ml. Because of high haemoglobin levels at birth, it is not practically possible to harvest the necessary blood volume to allow proper *in vitro* plasma binding determinations. *In vitro* studies are also influenced by other various factors (e.g. pH and temperature)[12] that further complicate such studies. Against this background, we decided to refrain from attempting to do *in vitro* analyses of plasma protein binding. It should be noted that eight of the AAGP concentration values in the material were slightly below the lower limit of quantification for the instrument used. Seven of these values were immediately below the limit (0.077–0.099).

Implications for dosing of LAs in newborn infants

The generally adopted guidelines for safe administration of LAs in children recommend a 40% reduction of the initial bolus and a 50% reduction of the maintenance infusion in neonates compared with older children.[21]

The variability in our material is substantial. This is mainly caused by the increasing concentrations of AAGP after 260 days of gestational age. However, during the period 220–260 days, the concentrations are reasonably stable at around 0.15 g litre^{-1}. Thus, an increased awareness for LAST and a further dose reduction (compared with the recommendations mentioned previously) could be used in patients under the gestational age of 260 days (36 weeks). Further reduction of the dose does not appear to be needed down to 220 days (27 weeks) according to our material.

Furthermore, when challenged with a neonatal surgical patient, not only pre- and postnatal ages need to be taken into consideration, but also the mode of delivery represents a factor in this context. Thus, slightly higher doses may be possible to use in infants born by normal vaginal delivery and acute Caesarean section than those born via elective Caesarean section. Our results also imply that even preterm infants are able to increase AAGP production in response to stressful events, and thus, should be able to increase their AAGP levels after surgery [e.g. surgical *patent ductus arteriosus* (PDA) closure].

We are currently looking further into this issue in extremely preterm infants undergoing surgical PDA closure. Better understanding of AAGP plasma concentrations in preterm and term infants will hopefully help to allow the safe use of continuous or intermittent boluses of LAs during the early postoperative period in this specific patient category. Furthermore, clearance of amide LAs is substantially lower in preterm and term infants, which may increase the risk for LAST further during continuous infusion.[22,23] Thus, the use of the ester LA, chloroprocaine, which is metabolised by plasma esterases and largely independent of liver maturation, has recently been suggested as a safer alternative.[24]

It is currently not possible to issue a more precise dosing recommendation based on our findings, but hopefully more studies in the field will lead to that. In conclusion, gestational age and mode of delivery need to be taken into consideration when determining the dosage of LAs in newborn infants.

Authors' contributions

Study design: M.A.-O., P.A.L., S.E., H.v.H., M.B.
Ethics application: M.A.-O.
Patient recruitment: M.A.-O., S.A.
Data collection: M.A.-O., S.A., M.B.
Data analysis: all authors.
Statistics: S.E.
Manuscript writing: all authors.
Senior supervision of the study: M.B.

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Declaration of interest

P.A.L. is an R & D advisory board member of Maquet Critical Care. The other authors have nothing to declare.

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