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Elina Kumpulainen

# *Central Nervous System Permeation of Non-Steroidal Anti-Inflammatory Drugs and Paracetamol in Children*

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UNIVERSITY OF
EASTERN FINLAND

**ELINA KUMPULAINEN**

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**ABSTRACT**

Non-steroidal anti-inflammatory drugs (NSAIDs) and paracetamol are widely used analgesics, acting in both the peripheral tissues and the central nervous system (CNS). However, knowledge on CNS permeation of these drugs in children in sparse. Therefore cerebrospinal fluid (CSF) penetration of diclofenac, ibuprofen, indomethacin, ketorolac, and paracetamol was studied in 160 healthy children (aged 3 months to 12 years) undergoing surgery with spinal anaesthesia. A single intravenous bolus dose of the study drug was given 10 minutes – 5 hours preoperatively, and a CSF sample was obtained during lumbar puncture for spinal anaesthesia. The concentration of drug in the CSF and in a paired plasma sample was determined by a gas chromatography-mass spectrometry method and by fluorescence polarization immunoassay.

After diclofenac 1 mg/kg, ibuprofen 10 mg/kg, indomethacin 0.35 mg/kg and ketorolac 0.5 mg/kg, the CSF concentrations ranged between 0.1 and 4.7 μg/l, 15 and 541 μg/l, 0.2 and 5.0 μg/l and 0.2 and 3.0 μg/l, respectively. The concentration ratios CSF/plasma were below 0.05, because of high (>99%) protein binding in plasma. The highest CSF concentrations of diclofenac, ibuprofen and ketorolac were detected an 1 hour after the injection, but indomethacin performed differently, with the highest concentrations in the CSF observed earlier (<30 min).

After paracetamol 15 mg/kg, the CSF concentrations ranged between 1.3 and 18.0 mg/l, with the highest concentrations at 1-2 hours. Paracetamol concentrations in the CSF reached and remained above the plasma concentrations after the first hour, because of low (<50%) protein binding of paracetamol in plasma.

In conclusion, indomethacin, ibuprofen, diclofenac, ketorolac and paracetamol permeate readily into the CSF in children. The peak concentrations after intravenous dosing are observed within an hour. However, there are differences between the drugs in both the timing of peak CSF concentrations and CSF/plasma ratios. These differences could impact on the speed of onset of analgesia and the toxicity profile of individual drugs.

National Library of Medicine (NLM) Classification: QV 38, QV 95, WL 203

Medical Subject Headings (Mesh): Acetaminophen; Central Nervous System/drug effects; Cerebrospinal Fluid/drug effects; Child; Child, Preschool; Diclofenac; Dose-Response Relationship, Drug; Ibuprofen; Indomethacin; Infant; Ketorolac; Pharmacokinetics, Time Factors; Tissue Distribution

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**TIIVISTELMÄ**

Tulehduskipulääkkeet ja parasetamoli ovat yleisesti käytettyjä kipulääkkeitä. Niiden vaikutus tapahtuu sekä perifeerisissä kudoksissa että keskushermostossa. Näiden lääkkeiden kulkeutuminen lasten keskushermostoon tunnetaan huonosti. Tässä tutkimuksessa selvitettiin diklofenaakin, indometasiinin, ibuprofeenin, ketorolaakin ja parasetamolin kulkeutumista aivo-selkäydinnesteeseen 160 terveellä lapsella (3 kk – 12 v), joille tehtiin leikkaus spinaalipuudutuksessa. Ennen puudutuksen pistämistä lapsille annettiin laskimoon yksi annos kipulääkettä. Spinaalipuudutuksen piston yhteydessä (10min – 5 tuntia lääkkeen annosta) otettiin aivo-selkäydinnestenäyte ja laskimoverinäyte. Näytteistä määritettiin lääkeainepitoisuus kaasukromatografia-massaspektrometria-menetelmällä ja fluoresenssi-polarisaatio-immuunimääritys-menetelmällä (parasetamoli-pitoisuus).

Diklofenaakin 1 mg/kg, indometasiinin 0,35 mg/kg, ibuprofeenin 10 mg/kg ja ketorolaakin 0,5 mg/kg antamisen jälkeen, aivo-selkäydinnesteen lääkeainepitoisuus vaihteli väleillä 0,1 – 4,7 μg/l, 15 – 541 μg/l, 0,2 – 5,0 μg/l ja 0,2 – 3,0 μg/l. Lääkeainepitoisuuksien aivo-selkäydinneste/plasma suhde oli alle 0.05, joka selittyy sillä, että plasmassa nämä lääkkeet sitoutuvat merkittävästi (>99 %) proteiineihin. Diklofenaakin, ibuprofeenin ja ketorolaakin korkeimmat pitoisuudet aivo-selkäydinnesteessä havaittiin tunnin kuluttua lääkkeen annosta, mutta korkeita indometasiinipitoisuuksia mitattiin aiemmin (<30 min).

Parasetamolin 15 mg/kg antamisen jälkeen, lääkeainepitoisuus aivo-selkäydinnesteessä oli 1,3-18,0 mg/l, ja korkeimmat pitoisuuden havaittiin tunnin kuluttua lääkkeen annosta. Parasetamolin pitoisuus aivo-selkäydinnesteessä saavutti saman tason kuin plasmassa tunnin kuluttua lääkkeen annosta, jonka jälkeen pitoisuudet aivo-selkäydinnesteessä ja plasmassa olivat samaa tasoa. Tämä selittyy parasetamolin vähäisellä (<50 %) sitoutumisella plasman proteiineihin.

Tässä tutkimuksessa todettiin, että indometasiini, ibuprofeeni, diklofenaakki, ketorolaakki ja parasetamoli kulkeutuvat keskushermostoon lapsilla, ja korkeimmat lääkeainepitoisuudet aivoselkäydinnesteessä havaittiin tunnin kuluttua laskimoannostelun jälkeen. Huippupitoisuuden ajankohdassa ja aivo-selkäydinneste/plasma pitoisuuksien suhteissa havaittiin kuitenkin merkittäviä eroja lääkeaineiden välillä, jotka voivat selittää erot eri lääkeaineiden vaikutuksen alkamisessa ja haittavaikutuksissa.

Luokitus: QV 38, QV 95, WL 203

Yleinen suomalainen asiasanasto (YSA): aivo-selkäydinneste, farmakokinetiikka, ibuprofeeni, indometasiini, keskushermosto, lapset, lääkeaineet – pitoisuus, parasetamoli, tulehduskipulääkkeet

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Kuopio, April 2010

Elina Kumpulainen

# *List of the original publications*

This thesis is based on the following original publications, which are referred to in the text by the Roman numerals I-V. Some unpublished data are also presented.

I Mannila A, Kumpulainen E, Lehtonen M, Heikkinen M, Laisalmi M, Salo T, Rautio J, Savolainen J, Kokki H: Plasma and cerebrospinal fluid concentrations of indomethacin in children after intravenous administration. J Clin Pharmacol. 2007;47(1):94-100.

II Kokki H, Kumpulainen E, Lehtonen M, Laisalmi M, Heikkinen M, Savolainen J, Rautio J: Cerebrospinal fluid distribution of ibuprofen after intravenous administration in children. Pediatrics. 2007;120(4):e1002-8.

III Kumpulainen E, Kokki H, Laisalmi M, Heikkinen M, Savolainen J, Rautio J, Lehtonen M: How readily does ketorolac penetrate cerebrospinal fluid in children? J Clin Pharmacol. 2008;48(4):495-501.

IV Kokki H, Kumpulainen E, Laisalmi M, Savolainen J, Rautio J, Lehtonen M: Diclofenac readily penetrates the cerebrospinal fluid in children. Br J Clin Pharmacol. 2008;65(6):879-84.

V Kumpulainen E, Kokki H, Halonen T, Heikkinen M, Savolainen J, Laisalmi M: Paracetamol (acetaminophen) penetrates readily into the cerebrospinal fluid of children after intravenous administration. Pediatrics. 2007;119(4):766-71.

# *Contents*

# *Abbreviations*

| | |
|---|---|
| AE | adverse effect |
| ASA | American Society of Anesthesiologists |
| AUC | area under the plasma drug concentration versus time curve |
| BBB | blood-brain barrier |
| BCSFB | blood-cerebrospinal fluid barrier |
| COX | cyclooxygenase enzyme |
| CNS | central nervous system |
| CP | choroid plexus |
| CSF | cerebrospinal fluid |
| CVO | circumventricular organs |
| DDD | defined daily dose |
| $ED_{50}$ | half maximal effective dose |
| EMEA | European Medicines Agency |
| FIMEA | Finnish medicines Agency (from 1.11.2009) |
| $IC_{50}$ | half maximal inhibitory concentration |
| im | intramuscular |
| ip | intraperitoneal |
| it | intrathecal |
| iv | intravenous |
| NAM | National Agency for Medicines (in Finland) (until 30.10.2009) |
| NRS | numeric rating scale |
| MRP | multidrug resistance-associated protein |
| NSAID | non-steroidal anti-inflammatory drug |
| OAT | organic anion transporters |
| OCT | organic cation transporters |
| OTC | over-the-counter |
| PACU | post-anaesthesia care unit |
| PC | plexus choroideus |
| PCA | postconceptional age |
| PG | prostaglandin |
| PGHS | prostaglandin H2 synthetase enzyme |
| po | per os |
| SPC | summary of product characteristics |
| teq | equilibration half-time (between plasma and the CSF) |

# 1 Introduction

Every year 5-10% of children undergo anaesthesia and surgery in Europe. Orthopaedic, gastrointestinal and oto-rhino-laryngological procedures are the most common operations in children (Clergue et al. 1999). Surgical procedures, anaesthesia and postoperative pain have a significant impact on the well-being of children (Hermann et al. 2006, Jones et al. 2009, Wollgarten-Hadamek et al. 2009). Postoperative pain in children is commonly managed with a multi-modal approach using paracetamol (acetaminophen), non-steroidal anti-inflammatory drugs (NSAIDs), opioids, and local anaesthetics (Kraemer and Rose 2009).

NSAIDs are used in infants and children older than 3 months to reduce pain, fever and inflammation, and in neonates to close the patent ductus arteriosus (PDA). They are commonly used for postoperative pain, because they reduce the need for opioids and increase patient satisfaction (Kokki 2003, Eustace and O'Hare 2007). However, NSAIDs may cause adverse effects, including gastric, renal and central nervous system (CNS) complications. Nevertheless, severe adverse effects are rare in short-term postoperative use in children. NSAIDs reduce pain by inhibiting the cyclooxygenase enzyme, both in the peripheral tissues and in the CNS.

Paracetamol is used in neonates and older children to reduce pain and fever, and it is important that the dose is large enough, especially in postoperative pain management (Anderson et al. 2001). In clinical use, adverse effects of paracetamol are rare, but unintentional high doses may cause liver toxicity. The actions of paracetamol seem to be mediated mainly in the CNS.

In order to have beneficial actions in the CNS, NSAIDs and paracetamol should permeate the CNS and achieve sufficient concentrations there to have an inhibitory effect on the central prostaglandin H2 synthetase (PGHS) enzyme, through which analgesic activity is mediated. However, the blood-brain barrier (BBB) regulates drug permeation in the CNS. The physico-chemical and pharmacokinetic characteristics of drugs have an effect on BBB permeation. The small molecular size and lipophilicity of NSAIDs and

paracetamol suggest that they may cross the BBB readily by diffusion (Davson and Segal 1996). However, extensive protein binding and ionization of NSAIDs may limit the amount of unbound drug available for permeation (Parepally 2005).

There are some adult studies on CSF permeation of NSAIDs and paracetamol (Bannwarth et al. 1990, Bannwarth et al. 1992, Rice et al. 1993, Bannwarth et al. 1995), but few paediatric studies. Ketoprofen has been studied in children with non-disturbed BBB (Kokki et al. 2002, Mannila et al. 2006), and paracetamol in children with intracranial pathologies (Anderson et al. 1998, van der Marel et al. 2003a). There are no previous studies of CSF permeation of indomethacin, ibuprofen, ketorolac, diclofenac and paracetamol in healthy children with a normal BBB. Therefore, this study was designed to evaluate the CSF permeation of indomethacin, ibuprofen, ketorolac, diclofenac and paracetamol in healthy infants and children aged 3 months to 12 years. An understanding of CSF pharmacokinetics in healthy children may help to understand both the onset time of analgesia and the toxicity profile of individual drugs.

# 2 Review of the literature

## 2.1 NSAIDS AND PARACETAMOL

### 2.1.1 History

The medical use of willow tree leaves and extracts, containing salicylate, dates back to 3000 BC. Willow was used to treat pain, fever and inflammation by the Assyrians, Babylonians and Egyptians (Mahdi et al. 2006). Acetylsalicylic acid (aspirin) was synthesised by Felix Hoffmann in 1897 and marketed by Bayer in 1900 (Mahdi et al. 2006). Paracetamol was synthesized in 1878, and marketed in the 1950s to replace phenacetin (Bertolini et al. 2006). Numerous new NSAIDs, such as indomethacin, ibuprofen and diclofenac, were prepared in the 1960s, and marketed shortly thereafter (O'Neil et al. 2001).

The mechanism of action of NSAIDs was discovered by Sir John Vane in 1971 (Vane 1971). After characterizing the different roles of housekeeping prostaglandin H2 synthetase-1 (PGHS-1, cyclooxygenase-1, COX-1) and inducible PGHS-2 (COX-2), there was an enormous commercial interest in the development of COX-2-selective agents, coxibs. Coxibs became popular because of their better gastrointestinal safety, but their use decreased after 2000 due to concerns about their cardiovascular safety (Helin-Salmivaara et al. 2006). Sometimes paracetamol is considered to belong to the group of NSAIDs. However, paracetamol has a different mechanism of action Anderson 2008), and it is often classified in a group of other analgesics and antipyretics.

### 2.1.2 Prevalence of use

NSAIDs and paracetamol are the most commonly used drugs worldwide. In Finland, the prevalence of over-the-counter (OTC) analgesic use among children aged 0-12 years is 7% (Ylinen 2008), which is only a little less than that in adults (Turunen et al. 2005). In children, the most commonly used OTC analgesic is paracetamol (Ylinen 2008), whereas ibuprofen is the most common analgesic in adults (NAM, Finnish Statistics on Medicines 01/2009-06/2009). In

Finland, paracetamol, naproxen and ibuprofen are the most commonly prescribed analgesics in children (Närhi and Kokki 2003).

In 2008, the pharmacy sales for NSAIDs were 86 DDD/1000 inhabitants/day, and for paracetamol 21 DDD/1000 inhabitants/day in Finland (NAM, Drug Consumption in 2005-2008). The use of NSAIDs increased 69 % during 1990-2007 and the use of paracetamol increased nine-fold during 1990-2007 (NAM, Finnish Statistics on Medicines 1990-2007). The use of NSAIDs and paracetamol has also increased in children (Närhi and Kokki 2003). In 2009, the use of NSAIDs remained the same, and paracetamol use has rose by 9% compared with the previous year (NAM, Finnish Statistics on Medicines 01/2009-06/2009).

## 2.2 MODE OF ANALGESIC ACTION OF NSAIDS

### 2.2.1 Prostaglandin H2 synthetase (PGHS) enzyme

In 1971 Sir John Vane discovered that the inhibition of prostaglandin (PG) synthesis is the mechanism of action for aspirin and other NSAIDs (Vane 1971). PGHS is the enzyme responsible for the metabolism of arachidonic acid to the unstable PGH2. The enzyme consists of two sites: a cyclooxygenase (COX) site and a peroxide (POX) site, which catalyse two reactions - a cyclooxygenase reaction in which arachidonic acid is converted to PGG2, and a peroxidase reaction in which PGG2 is converted to PGH2. PGH2 is further transformed by different prostaglandin synthetases to PGs (PGE2, PGD2, PGF2R, PGI2) and thromboxane A2 (TxA2) (Figure 1). PGs activate different G-protein coupled prostanoid-receptors, which affect cyclic adenosine monophosphate (cAMP), protein kinase c (PKC) and intracellular calcium, potassium and sodium concentrations (Svensson and Yaksh 2002, Tsuboi et al. 2002).

Two isoforms of the PGHS have been characterized. PGHS-1 (COX-1) and PGHS-2 (COX-2) are 60% homologous enzymes, but are coded by different genes in different chromosomes (loci 9q32-33.3 and 1q25.2-25.3, respectively). PGHS-1 is regarded as a housekeeping enzyme, acting constitutively in almost all cells and producing PGs that regulate physiological functions. PGHS-2 is

considered to be an inducible enzyme related to inflammation and pathological conditions, expressed in response to cytokines, mitogens, endotoxins and tumor promoters (Tanabe and Tohnai 2002, Blobaum and Marnett 2007). However, this seems to be too simple a viewpoint, since PGHS-2 is physiologically expressed in the brain, kidneys and reproductive tissues (Patrignani et al. 2005), and PGHS-1 expression increases in response to surgery (Zhu et al. 2003).

### 2.2.2 COX selectivity

Aspirin binds covalently to the COX site of the PGHS enzyme, whereas other NSAIDs are competitive COX inhibitors. Different NSAIDs have different affinity to COX-1 and COX-2. Some NSAIDs (for example, indomethacin and ibuprofen) are relatively unselective as they inhibit both COX-1 and COX-2 at similar concentrations; and the newest NSAIDs (coxibs; for example, celecoxib) are COX-2 selective, as they inhibit COX-2 in lower concentrations than COX-1. The selectivity of NSAIDs is expressed as a ratio of inhibitory concentration 50% ($IC_{50}$) for COX-1 and $IC_{50}$ COX-2. $IC_{50}$ is defined as the concentration of a drug, which is required for 50% inhibition of the enzyme or process. The selectivity of different compounds has been variable in different studies, since assays have different substrate concentrations, incubation times and protein concentration, and they may contain total cells, broken cells or enzymes from different species. Human whole blood assay is nowadays considered a standard (Tables 1-2) (Mitchell et al. 1993, Cryer and Feldman 1998, Warner et al. 1999).

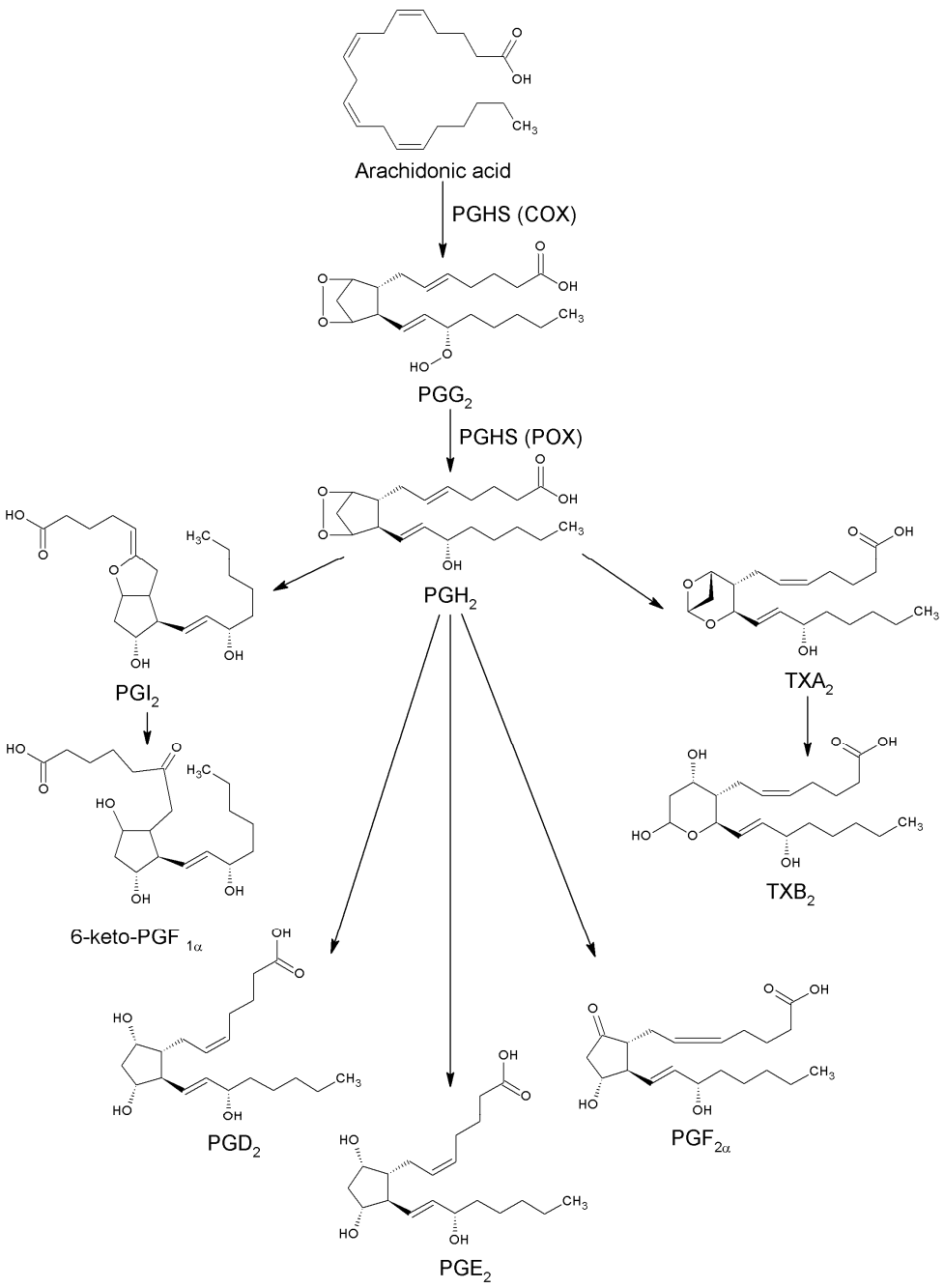


Figure 1. The arachidonic acid cascade, redrawn and modified from Vane et al. (1998)

Table 1. COX-selectivity of the analgesics investigated

| | **$IC_{50}$ COX-2/1 human whole blood assay*** | **$IC_{80}$ COX-2/1 human whole blood assay*** | **$IC_{50}$ COX-2/1 cultured, intact bovine aortic endothelial cells¤** | **$IC_{50}$ COX-2/1 human whole blood assay°** |
|---|---|---|---|---|
| **Indomethacin** | 80 | 11 | 60 | 1.78 |
| **Ibuprofen** | 0.9 | 1.2 | 15 | 1.69 |
| **Ketorolac** | 453 | 1176 | - | 0.68 |
| **Diclofenac** | 0.5 | 0.27 | 0.7 | 0.05 |
| **Paracetamol** | - | - | 7.4 + | 0.25 |

+ $IC_{30}$ ratio

* (Warner et al. 1999) ¤ (Mitchell et al. 1993) ° (Cryer and Feldman 1998)

Table 2. $IC_{50}$ (μg/l) in different study settings, of the analgesics investigated

| | **human whole blood assay** | | **human monocytes** | | **cultured, intact bovine aortic endothelial cells** | |
|---|---|---|---|---|---|---|
| | COX-1* | COX-2* | COX-1° | COX-2° | COX-1¤ | COX-2¤ |
| **Indomethacin** | 4.7 | 358 | 3.2 | 110 | 10 | 600 |
| **Ibuprofen** | 1 600 | 1 500 | 2 500 | 16 000 | 1 000 | 15 000 |
| **Ketorolac** | 0.048 | 22 | - | - | - | - |
| **Diclofenac** | 22 | 11 | 22 | 7.7 | 500 | 350 |
| **Paracetamol** | >15 000 | 7 400 | - | - | 2 700 + | 20 000 + |

+ $IC_{30}$ ratio

* (Warner et al. 1999), ° (Kato et al. 2001) and ¤ (Mitchell et al. 1993)

### 2.2.3 Peripheral site of action

Traditionally, NSAIDs are considered to be peripherally acting analgesics, because i) NSAIDs reduce PG synthesis, ii) peripheral tissue PG concentrations rise following trauma and inflammation, and iii) peripherally injected PGs increase vascular permeability and sensitize peripheral nociceptors, resulting in oedema, allodynia and hyperalgesia (Ebersberger et al. 1999). As further evidence for the peripheral site of action, topically applied NSAIDs have been shown to be effective, although inferior to systemic NSAIDs, in some osteoarthritis (Lin et al. 2004), soft tissue injury (Galer et al. 2000, Whitefield et al. 2002) and postoperative pain studies (Alessandri et al. 2006). It seems that percutaneous formulations reduce PG concentrations in inflamed tissues with minimal systemic exposition. In low pH-induced cutaneous pain, ibuprofen gel analgesia was not inferior to systemic ibuprofen. Ibuprofen concentrations at the peripheral injury site were similar, although ibuprofen plasma concentrations were 62 ng/ml and 25 μg/ml, after cutaneous gel and systemic drug, respectively (Steen et al. 2000). Moreover, there is evidence for a clinically relevant peripheral analgesic action of intra-articular NSAIDs in postoperative pain (Rømsing et al. 2000).

### 2.2.4 Central site of action

In addition to peripheral mechanisms, NSAIDs also have a central site of action, confirmed in numerous rodent studies. After intrathecal NSAID injection near the spinal cord, reduced pain-related behaviour is observed, dose-dependently and synergistically with morphine. Moreover, PGHS-1 and PGHS-2 –enzymes are found constitutively in the spinal cord, dorsal root ganglia and spinal dorsal and ventral grey matter; CSF prostanoid concentrations rise in response to peripheral injury or inflammation; and intrathecal prostanoid injections evoke hyperalgesia (Svensson and Yaksh 2002).

The central component has been estimated to account for 40% of the total analgesic efficacy for diclofenac (Burian et al. 2003), and has been suggested to be more sensitive than the peripheral site of action for ketorolac (Gordon et al. 2002). Moreover, it seems that spinal PGHS-1 and PGHS-2 have different roles in different pain states. It seems that spinal PGHS-2 is more involved in

inflammatory pain, whereas spinal PGHS-1 is more involved in the initial nociceptive pain (Zhu et al. 2003).

### 2.2.5 Inflammatory pain

Several studies confirm the role of spinal PGHS-2 in inflammatory pain. Firstly, the amount of spinal PGHS-2 and PGE2 increases in inflammatory pain. Secondly, this PGE2 release is blocked by intrathecal and systemic, selective COX-2 and unselective COX-inhibitors. Thirdly, the antihyperalgesic, dose-dependent, stereospescific effects of intrathecal COX-2 inhibitors, but not COX-1 inhibitors in inflammatory pain models have been proved. Fourthly, the antinociceptive activity (half maximal effective dose, $ED_{50}$) of several intrathecal COX inhibitors is correlated to their in vitro potency in blocking COX-2 ($IC_{50}$). Fifthly, drugs given by a spinal route are 100-500 times more potent than when given systemically (Malmberg and Yaksh 1992, Svensson and Yaksh 2002). It seems that PGE2-receptor EP2 subtype is the key mediator in spinal hyperalgesia followed by peripheral inflammation, but other PG receptors are involved in hyperalgesia after peripheral nerve injury and formalin injection (Reinold et al. 2005, Hösl et al. 2006). In rats after formalin injection, according to an experimental pain model protocol, the antinociceptive potency of intrathecal versus systemic (intraperitoneal) NSAIDs has been evaluated by Malmberg and Yaksh (1992) and Björkman (1995) (Table 3).

### 2.2.6 Nociceptive pain

Spinal PGHS-1 seems to play a major role in postoperative pain. In rats after paw surgery, PGHS-1 expression increases in the ipsilateral L4-L6 spinal dorsal horn, especially in the medial part, and in the gracile nucleus. In rats, intrathecally administered COX-1 inhibitors, but not COX-2 inhibitors, effectively reduce mechanical hypersensitivity after paw surgery (Zhu et al. 2003, Zhu et al. 2005) and restore normal behaviour after laparotomy (Martin et al. 2006).

Table 3. Half maximal effective doses ($ED_{50}$) of intrathecal and systemic analgesics in animal models of inflammatory pain

| | $ED_{50}$ (µg) | | Potency ratio |
|---|---|---|---|
| | **it** | **ip** | **ip vs it** |
| **Indomethacin *** | 0.68 | 930 | 807 |
| **S(+) Ibuprofen *** | 3.2 | NT | NT |
| **R (-) Ibuprofen *** | >56 | NT | NT |
| **Racemic ibuprofen *** | 3.9 | NT | NT |
| **Ketorolac *** | 1.3 | 770 | 216 |
| **Diclofenac ¤** | 3 | 300 | 100 |
| **Acetaminophen *** | 39 | 910 | 23 |

it intrathecal
ip intraperitoneal
NT not tested
* rat formalin test (Malmberg and Yaksh 1992) ¤ rat writhing test (Björkman 1995)

### 2.2.7 Intrathecal administration

In rats, intrathecal COX-1 inhibitors perform well in postoperative pain with minimal systemic exposure. Therefore, the desire to administer NSAIDs intrathecally to humans has arisen. The safety and efficacy of intrathecal ketorolac infusion has been studied in dogs and rats (Yaksh et al. 2004). Safety has been studied in healthy human volunteers in a dose-ranging phase I-study (Eisenach et al. 2002). However, the present commercially available ketorolac or other NSAIDs should not be given intrathecally because of potentially neurolytic excipients (alcohol, preservatives) and potential microbiological impurity.

### 2.2.8 Other targets besides COX

NSAIDs may also affect other targets besides the PGHS enzyme. Interactions with central opioid, serotonergic and nitric oxide systems have been described (Björkman 1995), although they may be indirect. Different NSAIDs may also directly inhibit and activate transcription factors, kinases and nuclear

receptors. Possible targets to different NSAIDs include nuclear factor kappa B, activator protein-1, MAP-kinase- family, protein kinase B, heat shock protein and peroxisome proliferator-activated receptor γ (Kankaanranta 1995). Some NSAIDs may have these COX-independent functions at concentrations which are attained in normal clinical use in humans.

### 2.3 MODE OF ANALGESIC ACTION OF PARACETAMOL

Whereas there is a consensus that NSAIDs exert most of their analgesic action by inhibiting the COX site of the PGHS enzyme, the mechanism of paracetamol action is not clear. Most commonly, paracetamol is considered to be a centrally acting PGHS-inhibitor (Flower and Vane 1972, Greco et al. 2003, Ayoub et al. 2006). Paracetamol is suggested to act as a reducing cosubstrate at the POX site of the PGHS enzyme. At the POX site, paracetamol reduces the amount of $Fe^{4+}$ (or $OPP^{*+}$) which is needed at the COX site for the generation of the tyrosine-385 radical which is needed in the cyclooxygenase reaction. The lack of anti-inflammatory and anti-thrombotic activity is explained by the swamping of POX with PGG2 and by the peroxide-tone with hydroperoxide-generating lipoxygenase enzymes (Graham and Scott 2005, Aronoff et al. 2006, Anderson 2008). Therefore, paracetamol is considered to have effects in the CNS with a strictly regulated microenvironment, but not in the periphery, in thrombocytes and in inflamed tissues.

Additionally, paracetamol may have effects on the brain serotonergic system (Graham and Scott 2005, Aronoff et al. 2006, Anderson 2008); in humans, pre-treatment with the 5-hydroxytryptamine (5-HT) antagonists tropisetron and granisetron seem to reduce the analgesic efficacy of paracetamol (Sandrini et al. 2003). Paracetamol may also affect the spinal L-arginine-nitric oxide system, because in animals pre-treatment with L-arginine but not with D-arginine reverses the pain behaviour induced by intrathecal N-methyl-D-aspartate and substance P (Björkman 1995). Paracetamol action by central nervous system (CNS) cannabinoid systems has also been suggested, as cannabinoid $CB_1$ receptor agonist completely prevents the analgesic activity of paracetamol (Bertolini et al. 2006). Moreover, paracetamol seems to be partly metabolized to AM404, which activates vanilloid subtype 1 receptor, which is a ligand of cannabinoid $CB_1$ receptor (Aronoff et al. 2006, Anderson 2008).

## 2.4 NSAIDS AND PARACETAMOL IN CLINICAL PRACTICE

### 2.4.1 Indications for NSAIDs

NSAIDs have multiple actions in the body, reducing fever, inflammation and pain. They have been approved for a wide variety of indications: in the treatment of fever, rheumatic diseases, and chronic and acute pain including cancer pain, dysmenorrhea, headache, dental, post-traumatic and postoperative pain. The widely used NSAIDs aspirin, ibuprofen and ketoprofen are available as OTC medications in Finland (FIMEA, NamWeb search).

Indomethacin and ibuprofen are used in preterm infants to close the patent ductus arteriosus (Van Overmeire and Chemtob 2005). Aspirin is used in the treatment of Kawasaki disease (Baumer et al. 2006).

### 2.4.2 Indications for paracetamol

Paracetamol reduces pain and fever, but lacks anti-inflammatory action. It has been approved for fever reduction and for treatment of chronic and acute pain.

### 2.4.3 Contraindications for and adverse effects of NSAIDs

Besides having beneficial effects on pain, fever and inflammation, NSAIDs have some important contraindications and adverse effects. These effects are common to all NSAIDs, because they are mediated by PGs. The normal regulation of physiological functions by PGs is altered with NSAID therapy, because COX inhibition reduces the formation of PGs. Most children tolerate NSAIDs well (Lesko and Mitchell 1995), but the contraindications and adverse effects should be taken into account when prescribing NSAIDs to children.

Constitutive COX-1 enzyme in the gastric mucosa produces PGs, which protect the integrity of the mucosa against gastric acid and enzymes. PGE and PGI increase the blood flow of mucosa, improve ulcer healing, increase the production of mucus and bicarbonate, and decrease the secretion of gastric acid. The effect of NSAIDs on gastric mucosa is also increased by ion trapping,

leading to intracellular accumulation of NSAIDs, which are weak acids. Gastric adverse effects are common in normal practice; at long-term use approximately 20% of patients suffer from nausea, abdominal pain, diarrhea, heartburn and gastrointestinal ulcers (Caruso and Bianchi Porro 1980). Patients at high age, with concomitant corticosteroid, selective serotonin reuptake inhibitor or antithrombotic medications and with previous gastrointestinal ulcers or with helicobacter pylori are at high risk of severe gastric adverse effects (Dalton et al. 2003, Hallas et al. 2006, Helin-Salmivaara et al. 2007). The risk of gastric adverse effects can be reduced by minimizing the daily dose and duration of NSAIDs therapy and by concomitant use of per oral PGE2, histamine-2 receptor antagonist or proton-pump inhibitor. The risk of gastric adverse effects is reduced by 50% when COX-2 selective NSAIDs are used instead of traditional agents (Hooper et al. 2004, Moore et al. 2006).

In normal situations, PGs have a minor role in maintaining renal function, but in patients with hypovolemia, hypotension, dehydration, cardiac insufficiency and renal disease PGs dilate renal blood vessels and help in maintaining normal renal blood flow and glomerular filtration. By blocking the PG production and vasodilatation, NSAIDs can cause acute renal failure, which is usually reversible. Moreover, minor, clinically irrelevant changes in renal function, and sodium and water retention occur commonly with NSAID therapy. Long-term use of NSAIDs may also cause interstitial nephritis and renal papillary necrosis. These renal adverse effects occur with both COX-2-selective and traditional NSAIDs at similar incidence (Whelton 1995, John et al. 2007, Lee et al. 2007).

Thromboxane A (TXA) causes thrombocyte aggregation and blood vessel vasoconstriction in the case of bleeding and vascular injury. Since NSAIDs also inhibit PG production by COX-1 in thrombocytes, they increase bleeding time. Therefore, all NSAIDs should be used cautiously in patients with bleeding disorders and anticoagulant medications (Rømsing and Walther-Larsen 1997, Cardwell et al. 2005). Moreover, COX-2 selective and traditional NSAIDs increase the risk of atherothrombosis, such as myocardial infarction. However, in patients with high risk of atherothrombosis, naproxen is assumed the safest NSAID (Helin-Salmivaara 2006, Kearney 2006).

In some asthmatics, NSAIDs cause changes in arachinoidic acid metabolism in the lungs. When the PG pathway is blocked by NSAIDs, AA is converted to cystein leukotriens, which cause bronchoconstriction and asthma attack. Some asthmatics (5-20%) are aspirin-intolerant, and cannot tolerate any NSAIDs (Lesko and Mitchell 1995, Lesko and Mitchell 1999, Lesko et al. 2002, Jenkins et al. 2004, Debley et al. 2005).

In fetuses and newborn infants, PGs inhibit the constriction of the musculature in the ductus arteriosus blood vessel wall (EMEA 2005, Van Overmeire and Chemtob 2005). Since NSAIDs inhibit the production on PGs, they may cause premature closure of the ductus arteriosus. Furthermore, PGs play a role in uterus contractions in normal labour, so NSAIDs may cause protracted labour. Moreover, NSAIDs may impair fertility and affect organogenesis. COX-2 inhibitors may have effects on glomerulogenesis (Kömhoff et al. 2000). Because of these effects, NSAIDs are contraindicated in pregnancy during the third trimester, and use of NSAIDs is avoided in all stages of pregnancy. Usually NSAIDs are not used in infants under 3 months age, and they are contraindicated in infants with ductus arteriosus dependent heart disease.

NSAIDs are contraindicated in severe liver insufficiency due to possible changes in metabolism and the risk of gastric adverse affects and bleeding (Davies and Anderson 1997, Kokki 2003). Moreover, the use of aspirin with concurrent viral infection (varicella zoster or influenza), may cause Reye syndrome, characterized by acute encephalopathy, hepatic steatosis and elevated levels of serum transaminases (Chow et al. 2003). Therefore, the use of aspirin is often avoided in children.

Occasionally NSAIDs cause central nervous system adverse effects such as drowsiness, headache, dizziness, vertigo and depression (Tharumaratnam et al. 2000, Clunie et al. 2003). With frequent use, NSAIDs may cause medication-overuse headache, which resolves after the withdrawal of analgesics (Diener and Limmroth 2004, Pakalnis et al. 2007). Skin reactions and other allergic reactions are rare after systemic NSAIDs, but common after topical NSAIDs (Lin et al. 2004).

### 2.4.4 Contraindications for and adverse effects of paracetamol

Paracetamol has fewer contraindications and causes fewer adverse effects than NSAIDs. Because of paracetamol metabolism in the liver and liver toxicity of metabolites, paracetamol is contraindicated in patients with severe liver insufficiency. Moreover, paracetamol should be used cautiously in patients with any hepatic disease, severe renal insufficiency or malnutrition, and hypovolemia should be corrected before use (Whelton 1995). Adverse effects, nausea, hypotension and allergic reactions, are rare. With frequent use, paracetamol may cause medication-overuse headache (Diener and Limmroth 2004, Pakalnis et al. 2007).

## 2.5 NSAIDS AND PARACETAMOL IN PAEDIATRIC POSTOPERATIVE PAIN

NSAIDs are widely used for postoperative pain in children older than 3 months (Eustace and O'Hare 2007). Paracetamol is the most commonly used analgesic in children at all ages, including in preterm neonates (Anderson 2004, Jacqz-Aigrain and Anderson 2006). NSAIDs and paracetamol are commonly combined in moderate and severe pain, because they may act synergistically and improve pain control (Viitanen et al. 2003, Hiller et al. 2006, Miranda et al. 2006, Salonen et al. 2009, Merry et al. 2010). Combining two NSAIDs increases the incidence of adverse effects, but not the efficacy (Kokki 2003).

In mild and moderate postoperative pain, NSAIDs and paracetamol as single agents or combined may perform well, with few adverse effects. In severe pain, NSAIDs and paracetamol are used as components of multi-modal analgesia, because of opioid-sparing effects and better patient satisfaction. Moreover, NSAIDs and paracetamol may reduce the incidence of opioid-related adverse effects such as pruritus, sedation, respiratory depression and vomiting (Kokki 2003, Anderson 2004, Jacqz-Aigrain and Anderson 2006). A decrease in the incidence and severity of vomiting has been observed in patients who have undergone tonsillectomy (Cardwell et al. 2005) and strabismus surgery (Kokki et al. 1999).

### 2.5.1 Dosing

The maturation of pharmacokinetics should be taken into account while prescribing NSAIDs and paracetamol to children. In newborn infants hepatic and renal clearance is reduced, total body water content is high, total body fat content is low and interindividual variation in pharmacokinetics is large, therefore reduced doses are needed. Most commonly hepatic clearance reaches adult values by the age of 6 months and renal clearance by the age of 2 years. Sometimes children at age of 6 months to 6 years need higher body weight adjusted doses, because hepatic clearance is increased (Bartelink et al. 2006, Kearns et al. 2010). However, in clinical work the dosing of most drugs, inclusing NSAIDs and paracetamol, is usually based on the body weight. The dosing of NSAIDs suggested by Kokki (2003) is presented in Table 4.

Paracetamol dosing is a controversial subject. An intravenous dose of 15 mg/kg three or four times per day and an oral dose of 15-20 mg/kg three times per day are commonly used (Table 5). Higher single oral doses of 40 mg/kg have also been studied (Anderson et al. 2001). In Finland the maximum recommended daily dose of paracetamol is 60 mg/kg divided in three or four doses over a day (FIMEA, NamWeb search).

Korpela and colleagues (1999) have shown that low dose suppositories (10 - 20 mg/kg) are ineffective in pain relief after surgery in children. In children who had undergone day-case surgery, $ED_{50}$ was 35 mg/kg, and the use of higher loading dose 40 mg/kg suppositories was suggested. In further studies it was shown that paracetamol absorption from suppositories is slow and erratic (Anderson 2004), which may account for poor pain relief in some children. However, in Finland the maximum recommended daily dose of paracetamol suppositories is 60 mg/kg. Because rectal bioavailability is low (0.3-0.98) (Montgomery et al. 1995, Anderson 2004), it can be argued that the maximum recommended daily dose of suppositories should be higher. Moreover, previous studies suggest that long-term use at doses above 60 mg/kg may cause liver damage, but short-term (up to 2 days) treatment (with doses less than 90 mg/kg/day) is safe, although large-scale studies are required to confirm this (Anderson et al. 2001, Hiller et al. 2006, Kozer et al. 2006).

Table 4. Dosing suggestions for some NSAIDs in paediatric postoperative pain (age > 3 months) (Kokki 2003)

| | **Single dose (mg/kg)** | **Dosing interval (h)** | **Maximum daily dose (mg/kg)** |
|---|---|---|---|
| **Indomethacin** | 0.35 | 6-8 | 2 |
| **Ibuprofen** | 10 | 6-8 | 40 |
| **Ketorolac** | 0.3-0.5 | 6-8 | 2 |
| **Diclofenac** | 1 | 8-12 | 3 |
| **Ketoprofen** | 1-2 | 6-8 | 5 |

Table 5. Dosing suggestions for intravenous paracetamol

| | **Loading dose** | **Maintenance dose** | **Doses per day** | **Maximum dose per day** |
|---|---|---|---|---|
| neonates<br>28-32 weeks PCA* | 20 mg/kg | 7.5 mg/kg | 3 | |
| neonates<br>33–36 weeks PCA* | 20 mg/kg | 7.5 mg/kg | 4 | |
| full-term neonates* | 20 mg/kg | 10–15 mg/kg | 4 | |
| full-term neonates, infants, children<br>weighing <10kg ¤ | 7.5 mg/kg | 7.5 mg/kg | 3-4 | 30 mg/kg |
| children<br>weighing 10-33kg ¤ | 15 mg/kg | 15 mg/kg | 3-4 | 60 mg/kg<br>2 g |
| children, adolescents<br>weighing 33-50kg ¤ | 15 mg/kg | 15 mg/kg | 3-4 | 60 mg/kg<br>3 g |
| children, adolescents, adults<br>weighing >50kg ¤ | 1 g | 1 g | 3-4 | 4 g |

PCA postconceptional age

*(Bartocci and Lundeberg 2007) ¤(Duggan and Scott 2009, SPC Perfalgan)

### 2.5.2 Formulation

In paediatric postoperative pain management, the correct formulation is especially important. In the immediate postoperative period, NSAIDs and paracetamol are best administered intravenously due to the improved dose accuracy (as mg/kg) and reliability, because the variability associated with enteral absorption is absent (Murat et al. 2005). After surgery and anaesthesia, in supine position, gastric emptying is delayed and vomiting is common, so oral formulations may have poor bioavailability or delayed absorption. Absorption from suppositories is sometimes variable and slow (Kokki et al. 2003, Anderson 2004, van der Marel et al. 2004, Kyllönen et al. 2005). Because of the costs of intravenous paracetamol, the loading dose of paracetamol is sometimes given by mouth before elective surgery (Anderson et al. 2001), or at high dose per rectum during anaesthesia or sedation (Korpela et al. 1999). However, as an application for marketing authorisation of generic intravenous paracetamol has been submitted, the costs of intravenous paracetamol are expected to decrease.

After recovery of gastric function, small or dispersing tablets or mixtures are the most preferred formulation. Intramuscular and rectal formulations are avoided in awake children, since children (Kokki 2003) and parents (Seth et al. 2000) dislike them. In Finland, intravenous formulations of ketorolac, diclofenac, ketoprofen and paracetamol are available. Paediatric paracetamol formulations include oral dispersing and normal tablets, oral mixture and suppositories. In addition, ibuprofen oral tablets and suppositories, as well as ketoprofen tablets are on the market in Finland (FIMEA, NamWeb search).

### 2.5.3 Adverse effects

NSAIDs rarely cause severe adverse effects in short-term postoperative use (Kokki 2003, Anderson 2004, Jacqz-Aigrain and Anderson 2006). Paracetamol rarely causes any adverse effects in short-term use in children when recommended doses are not exceeded.

*Bleeding*

NSAIDs may increase operative site bleeding because of effects on platelet aggregation. However, meta-analysis has shown that NSAIDs do not increase the risk of bleeding in healthy children without bleeding disorders or anticoagulant medications, (Rømsing and Walther-Larsen 1997, Cardwell et al. 2005). In any case, NSAIDs are commonly administered only after the achievement of primary haemostasis, especially after surgery with a significant risk of haemorrhage. Paracetamol may have minor effects on the bleeding time (Niemi et al. 2000, Munsterhjelm et al. 2005), but these effects are considered insignificant in most cases.

*Asthma*

NSAIDs may provoke asthma attacks in sensitive asthmatics. Asthmatics with nasal polyps are especially prone to asthma exacerbation. However, the risk of an asthma attack is 0.5-5 % if the asthmatic child does not have a history of NSAID-provoked broncho-constriction (Lesko and Mitchell 1995, Lesko and Mitchell 1999, Lesko et al. 2002, Jenkins et al. 2004, Debley et al. 2005). Paracetamol is well tolerated in aspirin-sensitive asthmatics; only approximately 7% of aspirin-sensitive asthmatics react to paracetamol (Jenkins et al. 2004).

## 2.6 DRUGS INVESTIGATED

### 2.6.1 Indomethacin

Indomethacin (Figure 2), an indole acetic acid derivative, is a traditional non-selective COX inhibitor. It has been available in Finland in an enteral form since 1965 and parenteral form since 1983 (FIMEA, NamWeb search). The marketing authorisation of intravenous formulation was withdrawn in October 2007 (FIMEA, NamWeb search). Indomethacin has been shown to be effective and safe in postoperative pain in children at age 1-16 years (Maunuksela et al. 1987, Maunuksela et al. 1988), but nowadays it is not commonly used in paediatric postoperative pain. Intravenous indomethacin is sometimes used for the closure of the PDA in preterm infants, but intravenous

ibuprofen has recently been marketed for that purpose (SPC Pedea, Ohlsson et al. 2008).

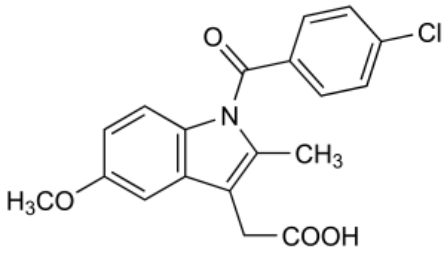


Figure 2. Indomethacin

*Pharmacokinetics*

Absorption of indomethacin after oral and rectal administration is complete and rapid, with peak plasma concentration 0.25 – 3hours (Helleberg 1981) (Table 6). It is highly (> 99.7%) bound to plasma proteins (Bannwarth et al. 1990). Indomethacin is subject to extensive enterohepatic recirculation. It is metabolized to O-desmethyl-metabolite by CYP2C9 (Nakajima et al. 1998) and to N-deschlorobenzyl-metabolite. The metabolites are conjugated with glucuronic acid and secreted in the urine and bile (Yeh 1985). The pharmacokinetics of has been studied in preterm neonates (Smyth et al. 2004, Al Za'abi et al. 2007), infants and children (Olkkola et al. 1989). The kinetics in children is similar to that in adults with an elimination half-life of 6 hours (Olkkola et al. 1989). Lower clearance and a longer elimination half-life of 20 hours is seen in preterm neonates, but clearance increases in the first postnatal six weeks and mature elimination half-life in reached (Smyth et al. 2004, Al Za'abi et al. 2007).

*Adverse effects*

Unique for indomethacin among NSAIDs is the high incidence of CNS adverse effects (AEs). Indomethacin commonly causes headache and dizziness, and rare cases of cognitive dysfunction, depression and psychosis have been reported (Tharumaratnam et al. 2000, Clunie et al. 2003). The mechanism by which indomethacin causes CNS AEs is unclear. Indomethacin has been shown to reduce cerebral blood flow in preterm infants (Mosca et al.

1997, Patel et al. 2000) and in healthy volunteers (Jensen et al. 1996). Either vasoconstriction or later observed vasodilatation in the brain may cause the CNS AEs. Furthermore, indomethacin and serotonin have structural similarities (indole-moiety). Therefore, CNS AEs may be caused by the direct effect of indomethacin on central neurons via the serotonin pathway, or a direct effect by some other mechanism, such as COX inhibition.

Table 6. Pharmacokinetic characteristics of the investigated drugs in adults (Avery's drug treatment, 1997)

| | **Oral bioavailability (%)** | **Total clearance (l/h, 70kg)** | **Half-life of the terminal elimination phase (h)** | **Apparent volume of distribution (l, 70kg)** |
|---|---|---|---|---|
| **Indomethacin** | <85 | 6.3 | 6 | 14 |
| **Ibuprofen** | <80 | 3.5 | 2.5 | 9.8 |
| **Ketorolac** | 80 | 2 | 5.6 | 17.5 |
| **Diclofenac** | 60 | 15.6 | 1.5 | 10.5 |
| **Paracetamol** | 70-90 | 19.3 | 2.5 | 65.8 |

### 2.6.2 Ibuprofen

Ibuprofen (Figure 3) is a chiral phenyl propionic acid derivative, a "profen", structurally similar to naproxen and ketoprofen, and non-selective COX inhibitor. It was launched in Finland in 1974 (FIMEA, NamWeb search), and since 1994 it has been the most commonly used analgesic in Finland (NAM, Finnish Statistics on Medicines 1990-2007). Ibuprofen is commonly used in postoperative pain management in children (Eustace and O'Hare 2007). It has been shown to be efficient after various operations, including tonsillectomy (Maunuksela et al. 1992b, Kokki et al. 1994, Pickering et al. 2002, Kokki 2003, Viitanen et al. 2003), in pain due to acute otitis media (Spiro et al. 2006), traumas (Clark et al. 2007), and in fever reduction (Lesko and Mitchell 1995, Goldman et al. 2004). Intravenous ibuprofen is used for the closure of the PDA (EMEA 2005, Ohlsson et al. 2008).

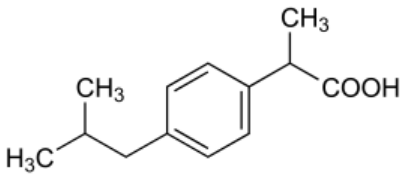


Figure 3. Ibuprofen

The stereoisomers of ibuprofen may have different actions (Evans 1996). It seems that S-ibuprofen but not R-ibuprofen elicits analgesic actions in the CNS, as demonstrated after intrathecal administration in rat formalin test and substance P-induced hyperalgesia (Malmberg and Yaksh 1992, Yaksh et al. 2001). Moreover, systemically given S-ibuprofen (dexibuprofen) has analgesic, antipyretic and anti-inflammatory actions. The actions of systemic R-ibuprofen are difficult to estimate, because unidirectional bioconversion of approximately 60% of the R to S isomer occurs (Kelley et al. 1992, Kyllönen et al. 2005).

*Pharmacokinetics*

Ibuprofen is completely and rapidly absorbed from different oral formulations, with peak plasma concentrations 0.25 – 3 h after administration. It is highly, > 99% bound to plasma proteins, mainly albumin. Ibuprofen is metabolised to inactive hydroxy and carboxy metabolites by CYP2C9 and CYP2C8, and partly conjugated with glucuronic acid. The metabolites are excreted mainly in the urine (Davies 1998). The elimination half-life of ibuprofen is 2 hours in children and adults (Davies 1998), but markedly prolonged elimination (30 hours) and decreased protein binding occurs in preterm and term neonates (Aranda et al. 1997, EMEA 2005, Hirt et al. 2008).

*Adverse effects*

Ibuprofen is considered to be one of the safest NSAIDs. Epidemiological studies have shown that short-term low-dose ibuprofen causes less gastro-intestinal complications than other NSAIDs (Henry et al. 1996, Hernandez-Diaz and Rodriguez 2000, Lewis et al. 2002). Moreover, large-scale studies show ibuprofen to be safe also in children and toddlers (Lesko and Mitchell

1995, Lesko and Mitchell 1999). However, the safety is dose-dependent and the incidence of adverse effects increases with increasing dose.

### 2.6.3 Ketorolac

Ketorolac (Ketorolac Tromethamine) (Figure 4) is a chiral pyrrole acetic acid derivative and a non-selective COX inhibitor. It has been marketed in Finland since 1991 (FIMEA, NamWeb search), and is indicated for the treatment of moderate and severe postoperative pain, for a maximum of two days (SPC Toradol). Ketorolac has been proved efficient in pain relief in children after various operations, including herniotomy and tonsillectomy (Forrest et al. 1997). The onset of analgesic action is slower, but is sustained longer than with morphine (Rice et al. 1991, Maunuksela et al. 1992a, Rice et al. 1995).

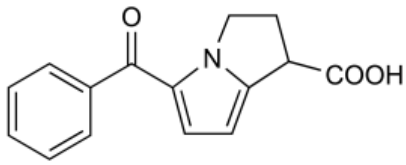


Figure 4. Ketorolac

Two stereoisomers of ketorolac seem to have different actions. S-ketorolac accounts for the analgesic and anti-inflammatory actions, while R-ketorolac is ineffective as an analgesic and anti-inflammatory agent and has no effect on COX (Mroszczak et al. 1996). Bioinversion R→S does not occur in humans, whereas inversion S→R does to some extent (6.5 %) (Mroszczak et al. 1996). However, there are major differences in inversion rates between different animal species, and inversion in the CNS has not been studied in humans or animals.

*Pharmacokinetics*

Ketorolac is completely and rapidly absorbed from intravenous, intramuscular and oral formulations. It is extensively (>99 %) bound to plasma proteins (Gillis and Brogden 1997). Ketorolac is metabolised by glucoronidation and para-hydroxylation. The metabolites (40%) and ketorolac (60%) are mainly excreted in the urine. Some age-related differences in

pharmacokinetics may occur, but the elimination half-life is similar (Olkkola and Maunuksela 1991, Forrest et al. 1997, Hamunen et al. 1999, Zuppa et al. 2009). The elimination half life is 5 hours in children and adults, but longer in the elderly and in patients with renal impairment (Gillis and Brogden 1997).

*Adverse effects*

The adverse effects associated with ketorolac are similar to those of other NSAIDs. Ketorolac is most extensively used in postoperative pain management, for short-term, in-hospital patients. The major concerns are operative site bleeding, renal adverse effects, upper gastrointestinal lesions/bleeding and allergic reactions. However, when patients with NSAID-related risk factors and adverse effects are excluded, the incidence of severe adverse effects is low, similar to other NSAIDs (Maunuksela et al. 1992a, DeAndrade et al. 1994, Forrest et al. 1997).

*Intrathecal administration*

Intrathecal administration of ketorolac has been studied in animals (Yaksh et al. 2004) and healthy volunteers (Eisenach et al. 2002). The study in rats and dogs confirmed the long term (1 month) safety of lumbar intrathecal ketorolac infusion (Yaksh et al. 2004). In a phase I dose-ranging human study, no adverse effects were noted (Eisenach et al. 2002). However, special preparations of ketorolac are required for intrathecal administration, because intravenously intended formulations may contain ethanol, preservatives and traces of bacteria and bacterial products.

### 2.6.4 Diclofenac

Diclofenac (Figure 5), a phenylacetic acid derivative, is a non-selective COX inhibitor. It has been available in Finland in enteral form since 1977 and parenteral form since 1984 (FIMEA, NamWeb search). In clinical use, intravenous diclofenac is added to buffered solutions, because there is a risk of supersaturation and crystal formation (SPC Voltaren). Diclofenac has been shown to be effective in postoperative pain. It reduces the need for morphine in children after appendicectomy (Morton and O'Brien 1999), strabismus

surgery (Wennström and Reinsfelt 2002), adenoidectomy (Baer et al. 1992) herniotomy and orchidopexy (Ryhänen et al. 1994).

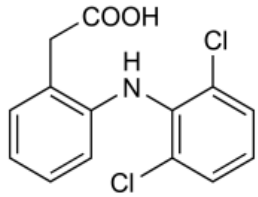


Figure 5. Diclofenac

*Pharmacokinetics*

Diclofenac is rapidly and completely absorbed from oral formulations (0.25 – 3 h and 90%), and from suppositories in adults and children (Davies and Anderson 1997, van der Marel et al. 2004). After oral absorption it undergoes first pass metabolism, with 60% of the drug reaching systemic circulation. Diclofenac is extensively (>99.7%) bound to plasma proteins, mainly albumin. Diclofenac is metabolized by CYP2C9 to hydroxyl metabolites and partly further conjugated to glucuronide and sulphate metabolites. The metabolites are excreted mainly in the urine (Davies and Anderson 1997). In adults and children the elimination half-life is 1 - 2 h (Korpela and Olkkola 1990, Davies and Anderson 1997).

*Adverse effects*

Diclofenac may cause adverse effects that are typical of all NSAIDs. However, adverse effects are rare in children in short-term use (Standing et al. 2009). Additionally, intramuscular injection of diclofenac rarely causes Nicholau syndrome, which is characterised by cutaneous and muscular necrosis at the site of injection. The aetiology of Nicholau syndrome has been suggested to involve intra- or peri-arterial drug injection, leading to ischemia of the skin and the muscle (Stricker and van Kasteren 1992, Ezzedine et al. 2004, Luton et al. 2006).

### 2.6.5 Paracetamol

Paracetamol (acetaminophen, Figure 6) belongs to the group of anilides. The oral formulation of paracetamol has been marketed in Finland since the 1960s (FIMEA, NamWeb search). Paracetamol is the most commonly used analgesic in many countries. In Finland, the use of paracetamol is rising (NAM, Finnish Statistics on Medicines 01/2009-06/2009 and 1990-2007). There are two intravenous paracetamol formulations available. Intravenous prodrug formulation (Pro-Dafalgan) contains propacetamol (2g), which is hydrolyzed rapidly to paracetamol (1g) by the plasma esterase. Another intravenous formulation (Perfalgan) containing paracetamol has been shown to be as effective as the prodrug formulation, but with less injection site pain (Murat et al. 2005). It has been marketed in Finland since 2002.

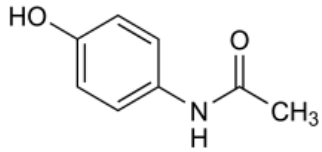


Figure 6. Paracetamol

Paracetamol has been shown to reduce the need for opioids and provide better pain management in children after various operations, including tonsillectomy (Anderson et al. 2001), herniotomy (Korpela et al. 1999, Murat et al. 2005) and orthopaedic operations (Granry et al. 1997). Some studies on postoperative pain relief have found non-superiority to placebo. The lack of pain relief in some studies is probably due to study design, such as opioid use and too low paracetamol doses (Hamunen and Kalso 2005, Korpela et al. 2007).

*Pharmacokinetics*

Paracetamol is rapidly and completely absorbed from oral formulations, whereas absorption from suppositories is erratic and slow (Montgomery et al. 1995, Anderson 2004). Degree of binding in blood is low (< 20%) (Milligan et al. 1994). Paracetamol is metabolized in the liver to glucuronide and sulphate conjugates, which are secreted in the urine. In adults, 60-80% and 20-30% of paracetamol is metabolized to glucuronide and sulphate conjugates,

respectively. In children, sulphate conjugation is more important and glucoronidation reaches adult levels only after birth (van der Marel et al. 2003b). A small part of paracetamol is metabolized through oxidation to hepatotoxic N-acetyl-p-benzoquinoneimine, which is conjugated with glutathione and further excreted in the urine as cysteine and mercapturic acid conjugates (Forrest et al. 1982). Additionally, minimal amounts of paracetamol are excreted in the urine unchanged. The elimination half-life is 3.5 hours in neonates, 1.5 – 2 hours in children (Anderson et al. 2005) and 2.5 hours in adults (Forrest et al. 1982, Duggan and Scott 2009). Clearance is reduced in preterm neonates when compared with term neonates (Table 5) (Allegaert et al. 2004a).

*Adverse effects*

The safety of paracetamol has been proved in large-scale studies, including in children (Lesko and Mitchell 1995 and 1999). The adverse effects (hypotension, nausea and elevated liver enzymes) are rare. Allergic reactions are very rare, and cases of blood count changes have been reported (SPC Perfalgan). Paracetamol may also cause kidney failure, especially in dehydrated patients (Whelton 1995).

Paracetamol may cause liver damage at overdoses (above 7.5 g in adults, and above 140 mg $kg^{-1}$ in children), because glutathione is depleted and toxic metabolite N-acetyl-p-benzoquinoneimine is bound to liver cells (Kozer et al. 2006). Treatment of overdose may include N-acetyl cysteine and liver transplantation (Hoppu 2002, Koivusalo et al. 2002, Kozer et al. 2006). Liver damage has been claimed to occur more common in alcoholics and malnourished patients (Prescott 2000). Overdoses may also cause nephrotoxicity, which is suggested to occur because of glutathione depletion in the kidneys (Boutis and Shannon 2001). Since paracetamol is available over-the-counter, poisoning is common in some countries. It is involved in approximately 50% of self poisonings in the UK (Camidge et al. 2003), and the annual incidence of paracetamol overdose requiring emergency department visit is 46 per 100 000 population in Canada (Myers et al. 2007). In Finland, serious paracetamol toxicity is rare (Isoniemi 2003).

## 2.7 BARRIERS BETWEEN BLOOD AND THE CENTRAL NERVOUS SYSTEM

The microenvironment of the CNS neurons is strictly regulated. Neurons are bathed in the interstitial fluid, which is separated from the cerebrospinal fluid by the ependyma. CNS fluid compartments are insulated from arterial blood by the BBB, the choroid plexus and the blood-spinal barrier. Circumventricular organs, regulating physiological functions such as the fluid balance, the circadian rhythm and vomiting, lack the BBB (Edwards 2001, Abbott 2004). The interfaces between fluid compartments in the CNS are shown in Figure 7.

### 2.7.1 History

Ehrlich and Goldmann were the first to recognize a barrier between blood and the central nervous system. Ehrlich (1885) noticed that intravenous blue dye stained the whole body except for the brain. Goldmann (1909) observed that intravenous trypan blue stained the meninges and the choroid plexus, but not the brain and the CSF. Moreover, Goldmann (1913) discovered that after intraventricular trypan blue injection, the whole brain was stained.

### 2.7.2 Definitions of the BBB and the BCSFB

The blood-brain barrier is defined as the site of exchange between blood, in the CNS capillaries, and the CNS extracellular fluid. The blood-cerebrospinal fluid-barrier (BCSFB) is defined as the site of exchange between blood in the capillaries and the CSF. One part of the BCSFB is the choroid plexus (CP).

### 2.7.3 Cerebrospinal fluid

Cerebrospinal fluid is a clear, colourless fluid contained within the ventricles and the subarachnoidal spaces around the CNS. CSF is formed by the CP, a leaf-like, highly vascular structure situated in the walls of the lateral ventricles and the roofs of the third and fourth ventricles. A smaller part of the CSF may be drained from the brain interstitial fluid after ependyma filtration. The rate of CSF formation in adults is approximately 500 ml per day (Davson and Segal 1996). The total intracranial volume of the CSF is 165 ml (range 62 - 267 ml) in young adults (Tanna et al. 1991). The volume of lumbosacral CSF is 36 ml

(range 11 – 61 ml) (Sullivan et al. 2006). When adjusted to body weight, the volume of intracranial CSF is constant in children older than 3 months (Pfefferbaum et al. 1994) and the rate of formation is similar to that in adults (Blomquist et al. 1986, Johnston and Teo 2000).

From the paired lateral ventricles, CSF flows via the foramina of Monro into the third ventricle. The third ventricle is connected via the aqueduct of Sylvius to the fourth ventricle, which opens via the foramen of Magendie and the foramina of Luschka to the subarachnoidal space. CSF in the subarachnoidal space surrounds the spinal cord inside the spinal canal, and caudally continues until the sacrum. CSF is drained mainly to the dural venous blood stream via valvular arachnoid granulations (Davson and Segal 1996), although a part may be drained to the lymphatic system (Koh et al. 2005).

### 2.7.4 Structure of the BBB

The BBB (Figure 8) consists of brain capillary endothelial cells, basement membrane, pericytes and astrocyte foot processes. Brain capillary endothelial cells are polarized, non-fenestrated endothelial cells, which are interconnected by tight junctions. Pericytes cover 20 – 30 % of the capillary surface and have a continuous basement membrane with brain capillary endothelial cells. Astrocytic endfeet project near the capillary (Davson and Segal 1996, Graff and Pollack 2004, de Boer and Gaillard 2007).

### 2.7.5 Structure of the CP

The choroid plexus (Figure 9) consists of capillary endothelial cells, stroma, basement membrane and choroid epithelium (ependyma). Each CP villus contains centrally packed blood vessels surrounded by stroma with pial cells and collagen fibrils. The capillary endothelial cells are polarized and fenestrated. Each villus is surrounded by choroid epithelium, which lays over a basement membrane. Choroid epithelium is a layer of tightly packed cuboidal epithelial cells, which are connected by tight junctions. Choroid epithelial cells contain cilias at the apical side, facing the CSF. The choroid epithelial cell layer is continuous with the ependyma, which lines the ventricles and separates the CSF from the brain (Davson and Segal 1996, Graff and Pollack 2004, de Boer and Gaillard 2007).

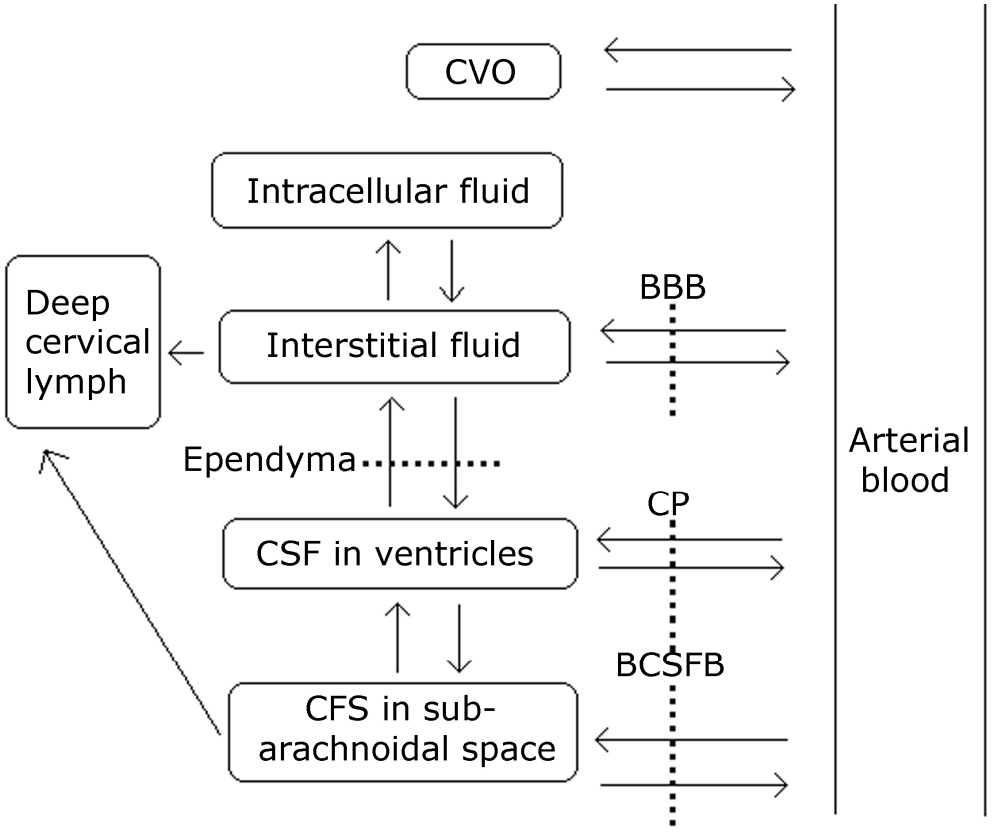


Figure 7. Diagram showing the interfaces between different fluid compartments in the central nervous system (CNS). Broken lines represent the barriers: the blood-brain barrier (BBB), the ependyma and the blood-cerebrospinal fluid-barrier (BCSFB), including the choroid plexus (CP). Th paths of fluid movement are marked with arrows. Circumventricular organs (CVO) are not separated from blood by barriers. Redrawn and modified from de Boer and Gaillard (2007).

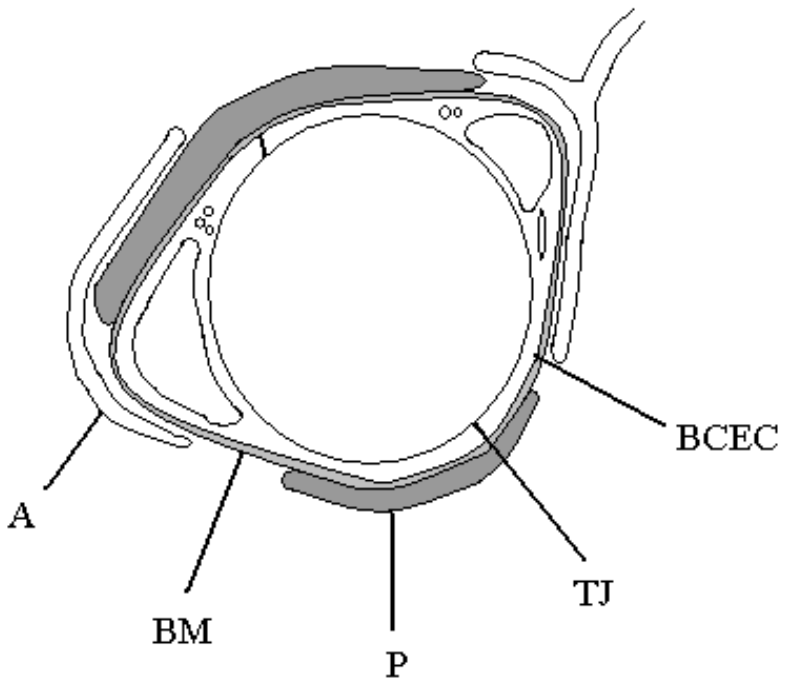


Figure 8. Schematic cross-section of the blood-brain barrier (BBB). Brain capillary lumen is surrounded by the brain capillary endothelial cells (BCEC) with tight junctions (TJ), pericytes (P), basement membrane (BM), astrocytic endfeet (A). Redrawn and modified from de Boer and Gaillard (2007).

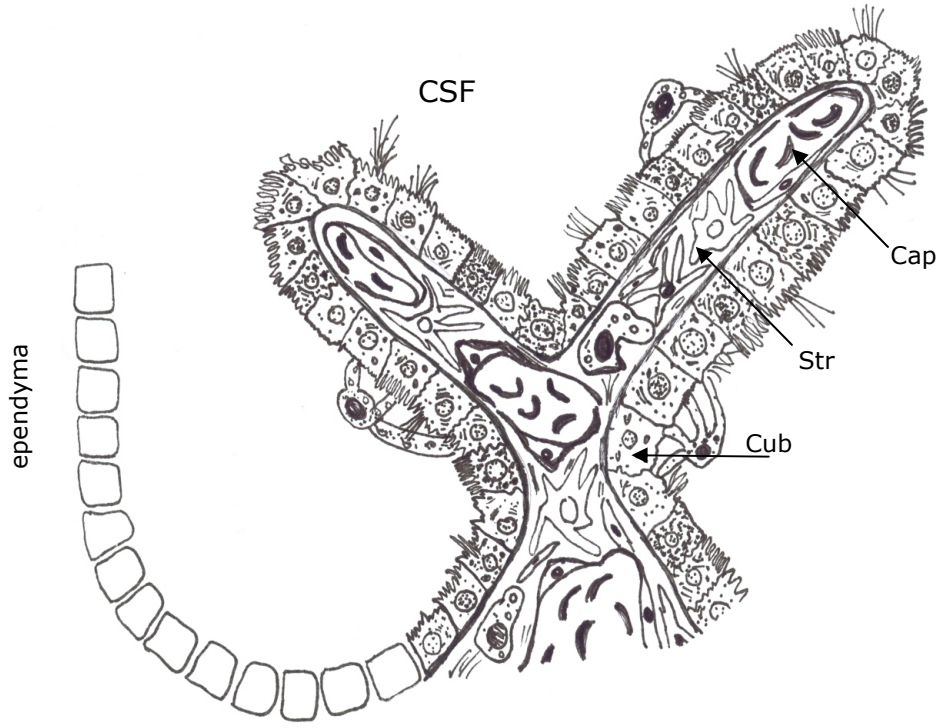


Figure 9. Schematic cross-section of the choroid plexus. Two choroid plexus villi contain a fenestrated capillary. The capillary (Cap) is surrounded by stromal connective tissue (Str), composed of collagen fibres, fibroblasts, dendritic cells and macrophages. The outer simple cuboidal epithelial cells (Cub) lie on a basement membrane. The cuboidal cells are connected by tight junctions and bear microvilli and cilia at their apical side. Kolmer cells (epiplexus cells) lie on the ventricular surface of the cuboidal cells. Redrawn and modified from Strazielle and Ghersi-Egea (2000).

### 2.7.6 Functions of the barriers

The name 'barrier' originates from the earliest experiments (Ehrlich 1885, Goldmann 1909), where dyes failed to pass from blood to the CNS. In further studies, the BBB and BCSFB have been shown to have more intricate regulatory actions, by which the CNS homeostasis is maintained. The barriers i) regulate ionic homeostasis, ii) restrict small hydrophilic molecule permeation, iii) facilitate influx and efflux transport by specific carriers, iv) restrict and regulate endocytotic permeation of large molecules, v) separate peripheral and central neurotransmitter pools and vi) provide immune privilege to the CNS (Davson and Segal 1996, Abbott 2004).

### 2.7.7 Immature barriers

It is widely believed that the BBB and CP are immature in neonates (Saunders et al. 1999). The schedule for the development of the barrier structure and function in humans has not been established (Saunders et al. 2000, Dziegielewska et al. 2001). It has been hypothesized that small lipophilic molecules may diffuse through the barriers more readily in the fetal brain than in the adult brain. It has also been suggested that the "sink action" increases with age and therefore brain bioavailability decreases with age.

Also other considerable central nervous system maturation occurs during the postnatal period, and physiological processes undergo maturation. The cerebral (cortical) metabolic rate increases postnatally until the age of 3-4 years when it reaches levels twice those observed in adults (Albert et al. 1999). Moreover, the cerebral blood flow is reduced in the first 6 months of life, then increases to peaks at age 3 to 4 years, and thereafter decreases to adult levels at the age of 9 years (Zwienenberg and Muizelaar, 1999). Additionally, the relative size and weight of brains is larger in younger and smaller children than bigger and older children and adults (Albert et al. 1999). These factors may have an effect on the central nervous system permeation of compounds, and therefore small children may have higher CNS bioavailability of some drugs than adults. However, knowledge on maturation of CNS pharmacokinetics in children is sparse.

### 2.7.8 Mechanisms of barrier permeation

Components may cross the BBB and the BCSFB by many mechanisms (Figure 10). Physicochemical properties and the pharmacokinetics of the drug determine whether the drug reaches the site of the BBB and the BCSFB. Therefore, absorption, distribution, metabolism and elimination have major impacts on barrier permeation. Furthermore, cerebral blood flow also has effects on BBB and BCSFB permeation (de Lange and Danhof 2002, de Boer et al. 2003).

*Transcellular diffusion*

Small non-charged lipophilic molecules may cross the barriers by passive transcellular diffusion (Davson and Segal 1996). Passive diffusion can be predicted by Abraham's method with 19 descriptors (Zhao et al. 2007) and by Lipinski's rule of five (Lipinski et al. 2001). Lipinski's rule (Lipinski et al. 2001) predicts poor permeation when there are more than 5 H-bond donors and more than 10 H-bond acceptors, when the molecular weight is more than 500 g $mol^{-1}$ and when the calculated LogP is greater than 5. The rule excludes compound classes that are substrates for biological transporters. In general, passive diffusion leads to equilibrium with drug in plasma and in the brain or the CSF. However, only the free concentration of a drug is available for diffusion so protein binding affects the amount of unbound drug available for diffusion.

*Paracellular diffusion*

Paracellular diffusion does not occur at the BBB or the BCSFB (Davson and Segal 1996). At the BBB, it is restricted by the tight junctions between brain capillary endothelial cells. At the CP, the fenestrated capillary endothelial cells permit it, but choroid epithelial cells restrict it by the tight junctions.

*Carrier-mediated transport*

Compounds may cross the BBB and BCSFB by carrier-mediated transport (Davson and Segal 1996, de Boer et al. 2003, Graff and Pollack 2004), which requires energy (Adenosine Triphosphate (ATP) -dependent) or transport of

another compound (symport or antiport). Influx transport systems facilitate the transport of many essential compounds in to the brain. There are specific transporters for glucose, ions and different types of amino acids. Efflux-transport mechanisms facilitate the elimination of metabolites and other components from the brain. Efflux transporters include P-glycoprotein (P-gp), multidrug resistance-associated protein (MRP), organic cation transporters (OCT) and organic anion transporters (OAT).

*Metabolic activity*

Cells at the BBB and the BCSFB express many metabolic enzymes, which act as a functional barrier (de Lange and Danhof 2002, Graff and Pollack 2004). Enzymes catalyzing phase I (cytochrome P450 (CYP), monoamine oxidase MAO, alcohol dehydrogenase family) and phase II reactions (UDP-glucuronosyltransferases UGTs, sulfotransferases STs, glutathione-S-transferases, GSTs) have been found in the brain capillary endothelial cells and at the CP (Ghersi-Egea and Strazielle 2001, Strazielle et al. 2004, Ghersi-Egea et al. 2006, Gradinaru et al. 2009).

*Endocytotic transport*

Endocytotic transport includes fluid-phase, adsorptive and receptor-mediated endocytosis (Davson and Segal 1996). In general, vesicular activity is down regulated at the BBB and BCSFB. However, transferrin, insulin, low-density lipoproteins and certain proteins cross the barriers by endocytosis.

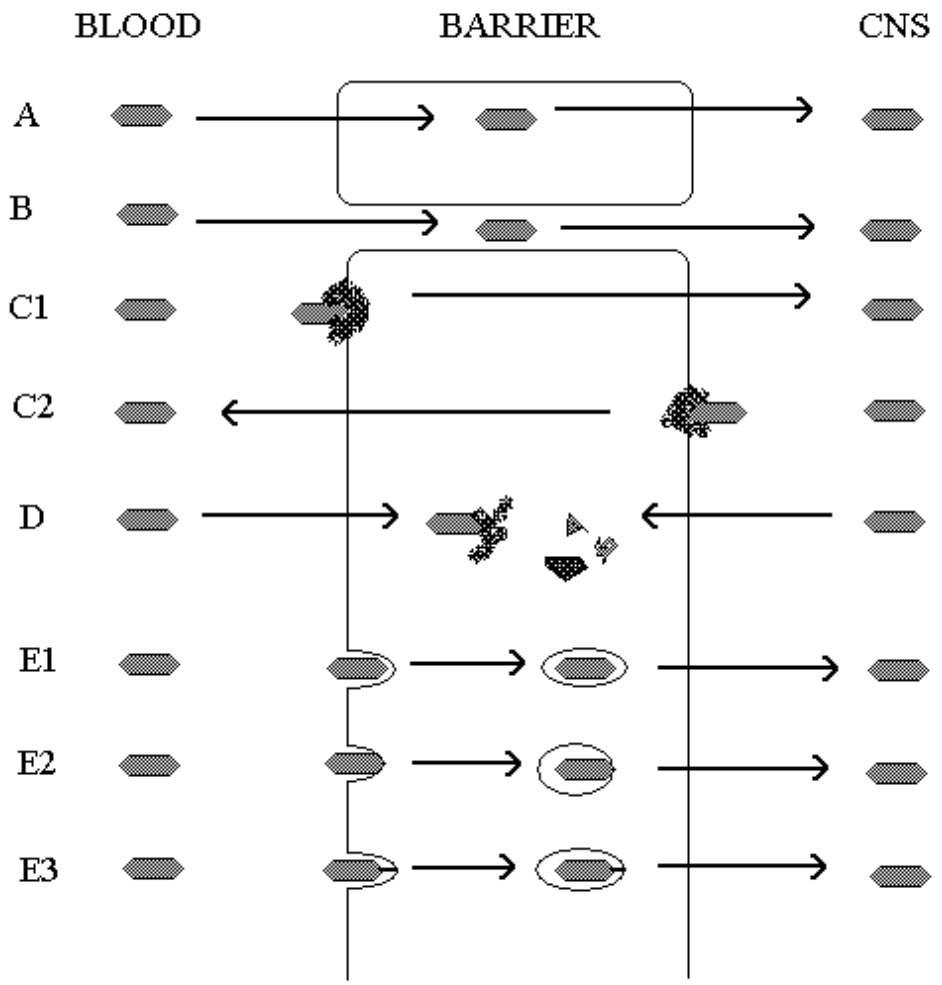


Figure 10. Diagram showing transport mechanisms across the barriers. A: transcellular diffusion. B: paracellular diffusion. C: carrier-mediated transport: C1: influx, C2: efflux. D: metabolism. E: endocytosis: E1: fluid-phase endocytosis, E2: adsorptive endocytosis, E3: receptor-mediated endocytosis. Redrawn and modified from Wolka et al. (2003).

## 2.8 PENETRATION OF NSAIDS AND PARACETAMOL INTO THE CNS

There are some human studies on NSAID and paracetamol penetration into the CSF. The results are summarized in this section.

### 2.8.1 Transcellular diffusion of NSAIDs

Some physico-chemical characteristics of the study drugs are presented in Tables 7-8. NSAIDs are small molecules with a molecular weight of 200 – 360 g/mol. The logP for NSAIDs is 2.7-4.4, and they have fewer than 5 H-bond donors and 10 H-bond acceptors. Therefore, Lipinski's rule of five (Lipinski et al. 2001) does not predict poor permeation.

NSAIDs are lipophilic molecules; their N-Octanol – water partition coefficient in pH 7.4 ($logD_{7.4}$) is 0.4 – 1.2. This would predict good permeation by diffusion. The relationship between drug lipophilicity and CSF diffusion was evaluated by Péhourcq and coworkers (2004). The discovered relationship was parabolic: at lipophilicity index log $k_{IAM}$ between 1.1 and 1.7, the drug entered the CSF easily; the CSF/plasma (AUC) ratio was above 1. With log $k_{IAM}$ less than 1.1 or above 1.7, the CSF/plasma ratio was below 1. The values log $k_{IAM}$ 1.1 and 1.7 represent logP values of approximately 2.64 and 4.25 (Péhourcq et al. 2003).

NSAIDs are weak acids and their pKa dissociation constants range from 3.5 to 4.9. Calculated with the Henderson-Hasselbalch equation, only 0.01 – 0.3 % of NSAIDs in plasma is in unionized form. This limits NSAID permeation, since only the unionized, non-charged fraction may diffuse through the barriers.

NSAIDs are extensively, more than 99%, bound to plasma albumin. Protein binding has a major effect on NSAIDs penetration in the CSF (Parepally 2005). Parepally studied brain concentrations in rats after in situ perfusion, at albumin levels matching 0, 1%, 10% and 100% of normal. He discovered that ibuprofen, indomethacin and flurbiprofen concentrations in the rat brain correlated well with free drug concentration in the plasma. Furthermore, young children (under 2 years old) have lower plasma albumin concentrations (Gomez et al. 1984), and therefore unbound NSAID concentrations in plasma may be higher and also result in higher CSF concentrations.

NSAIDs may bind to proteins, mainly to albumin, also in the CSF. The albumin concentration in the CSF is low, approximately 0.5% that of plasma (ISLAB, 2009). Protein binding in the CSF results in higher concentration levels, since the unbound plasma drug reaches equilibrium with the unbound CSF concentration. There is one previous study on indomethacin binding to proteins in the CSF (Müller et al. 1991). Müller and colleagues studied the binding of indomethacin to CSF proteins by equilibrium dialysis, after adding indomethacin to pooled CSF samples from ten patients. They found that indomethacin is 40% bound to albumin in the CSF. Furthermore, the protein binding of NSAIDs in the CSF can be extrapolated from the plasma data by using binding equations. Additionally, young children (under the age of 4-6 months) have higher CSF protein concentrations, and therefore may have higher total concentrations of NSAIDs in the CSF (Biou et al. 2000, Wong et al. 2000).

### 2.8.2 Transcellular diffusion of paracetamol

Paracetamol is a small molecule. The molecular weight, 150 g/mol, is half that of NSAIDs. The logP for paracetamol is 0.5, and it has less than 5 H-bond donors, and 10 H-bond acceptors. Therefore, Lipinski's rule of five (Lipinski et al. 2001) does not predict poor permeation.

Paracetamol is lipophilic and it has a $logD_{7.4}$ of 0.4. It is a weak acid, which is 99 % unionized in plasma, making it capable of diffusing the barriers. Paracetamol is has a low degree of binding to erythrocytes and plasma proteins at therapeutic concentrations (<20%) (Milligan et al. 1994), which also suggests good availability for permeation to the CNS.

Table 7. Physicochemical characteristics of some NSAIDs and paracetamol (NLM ChemIDplus)

| | molecular weight (g/mol) | Number of H-bond donors | Number of H-bond acceptors |
|---|---|---|---|
| **Indomethacin** | 358 | 1 | 4 |
| **Ibuprofen** | 206 | 1 | 2 |
| **Ketorolac** | 255 | 1 | 4 |
| **Diclofenac** | 296 | 2 | 3 |
| **Ketoprofen** | 254 | 1 | 3 |
| **Paracetamol** | 151 | 2 | 2 |

Table 8. Physicochemical characteristics of some NSAIDs and paracetamol: pKa dissociation constant, unionized drug percent in plasma pH 7.4, logP (intrinsic partition coefficient determined at pH 2.0) and $logD_{7.4}$ (apparent partition coefficient determined at pH 7.4)

| | pKa Dissociation Constant * | Unionized drug in pH=7.4 (%) | logP | logD7.4 |
|---|---|---|---|---|
| **Indomethacin** | 4.5 | 0.13 | 4.27¤ | 0.91 ¤ |
| **Ibuprofen** | 4.91 | 0.32 | 3.50¤ | 1.07 ¤ |
| **Ketorolac** | 3.49 | 0.012 | 2.74 + | 0.40 + |
| **Diclofenac** | 4.15 | 0.056 | 4.40¤ | 1.22 ¤ |
| **Ketoprofen** | 4.45 | 0.11 | 3.12¤ | -0.25¤ |
| **Paracetamol** | 9.38 | 98.95 | 0.4 * | 0.4 a |

a. because paracetamol is 98.95% unionized in plasma pH=7.4, $logD_{7.4} \approx logP$
*(NLM PubChem), ¤(Barbato et al. 1997)), +(Jett et al. 1999).

### 2.8.3 Interaction with transport systems

There are few studies on NSAID and paracetamol interactions with transporter systems. There is evidence of ibuprofen, indomethacin and ketoprofen transport by organic anion transporters (OAT1 and OAT3) in cell cultures (Khamdang et al. 2002). Diclofenac but not paracetamol BBB permeation in rats has been suggested to involve a transporter such as OAT3 (Fukuda et al. 2005). Both OAT1 and OAT3 are present at the BBB and the BCSFB as apical efflux proteins (de Boer et al. 2003, Graff and Pollack 2004). The BBB ibuprofen transport has been shown to be in part self-saturable, and inhibited by indomethacin in rats (Parepally et al. 2006). Parepally et al. suggest that ibuprofen transport in the brain involves many transporters or an as yet unidentified transporter. They found no evidence of a saturable component for either indomethacin or flurbiprofen BBB transport. NSAIDs may also interact with multidrug resistance associated protein (MRP) and organic cation transport protein (OCT) action (Khamdang et al. 2002, Reid et al. 2003), which have been found at the BBB and the BCSFB facilitating efflux (MRP and OCT) and uptake (OCT) actions (de Boer et al. 2003, Graff and Pollack 2004).

### 2.8.4 Indomethacin

Indomethacin concentrations in the CSF have been evaluated by Dittrich et al. (1984) and Bannwarth et al. (1990). Dittrich and coworkers (1984) detected no indomethacin in the CSF, probably because their assay had a high lower limit of quantification (50 μg/l). Bannwarth and coworkers (1990) administered an intramuscular injection of 50 mg indomethacin (0.72 mg/kg) to 52 adult patients who underwent lumbar puncture for myelography. Indomethacin concentrations in the CSF ranged between 1.0 and 11.9 μg/l at 30 minutes to 12 hours. Two high observations of indomethacin concentrations in the CSF at 2 and 4 hours have a major effect on the means of the concentrations (Figure 11). Therefore, it seems that the peak values (5 μg/l) occurred at 1 to 4 hours after administration. The median of ratio CSF to total plasma was 0.0048 and the median of the ratio CSF to free plasma was 1.68. The ratio CSF to free plasma was above 1 in all samples at 3 to 12 hours after administration.

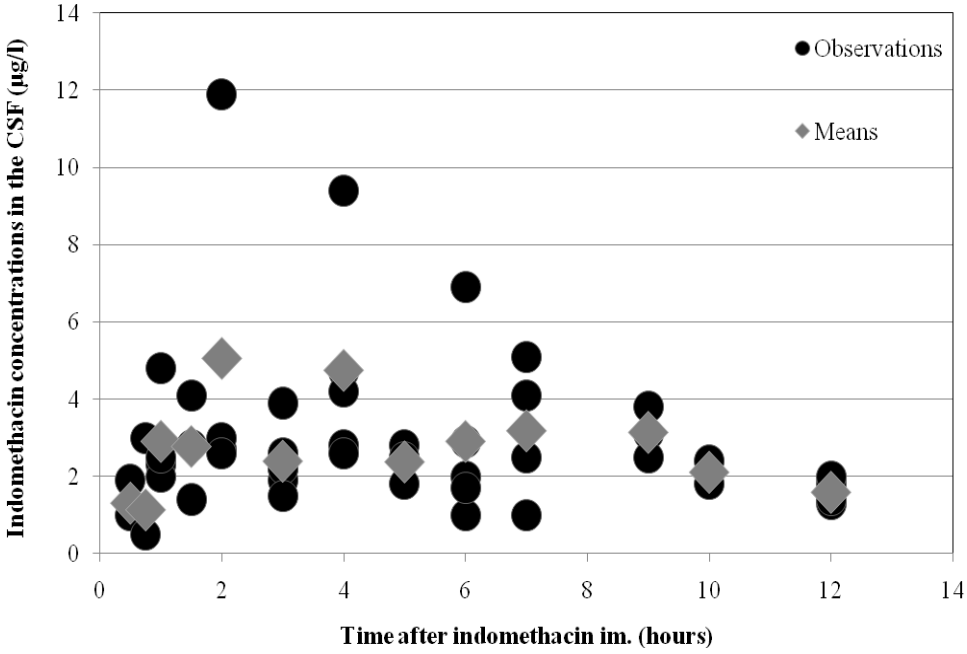


Figure 11. Indomethacin concentrations in the CSF in 52 adults after intramuscular indomethacin 50 mg (0.72 mg/kg) (Bannwarth et al. 1990). There are two high concentrations at 2 and 4 hours, which skew the mean observations at those times.

### 2.8.5 Ibuprofen

Penetration of ibuprofen enantiomers in the CSF has been studied by Bannwarth and coworkers (1995). They administered an oral dose of 800 mg racemic ibuprofen (corresponding to 11.4 mg/kg) to 46 adult patients who were suffering from nerve-root compression pain and were undergoing myelography. Ibuprofen was undetectable in samples at 30 minutes, but peaked at 3 hours at mean concentrations of 168 and 315 µg/l for R(-) and S(+) ibuprofen, respectively. The CSF concentrations exceeded unbound plasma concentrations at 1.5 to 8 hours. The AUC CSF to total plasma ratio was 0.009 and 0.15 for the R(-) and S(+) ibuprofen, respectively.

### 2.8.6 Ketorolac

Ketorolac concentrations in the CSF have been studied by Rice and coworkers (1993). Altogether 29 adult patients undergoing spinal anaesthesia received intramuscular ketorolac 90 mg (corresponding to 1.2 mg/kg). Ketorolac concentrations in the CSF ranged between 0.4 and 5.7 μg/l (median 2.1 μg/l) at 1 to 4.5 hours. The ratio of CSF to total plasma was 0.00007 – 0.00182 (0.00034).

### 2.8.7 Diclofenac

Diclofenac concentrations in the CSF have been measured in two adult patients after intramuscular injection of diclofenac 75 mg (Zecca et al. 1991). The CSF concentrations were 3.54 and 8.50 μg/l, at 2 and 12 hours, respectively. The concentration ratios of CSF to total plasma were 0.003 and 0.08.

### 2.8.8 Other NSAIDs

The penetration of ketoprofen in the CSF has been studied in children (Mannila et al. 2006, Kokki et al. 2002) and adults (Netter et al. 1985). Netter and coworkers (1985) administered intramuscular ketoprofen 100 mg to 36 adults suffering from sciatica. They detected ketoprofen in the CSF at 15 minutes to 13 hours after drug administration, with a peak at 3-5 hours. From the 2$^{nd}$ to the 13$^{th}$ hour the CSF concentrations were in equilibrium with the free plasma levels.

Kokki and coworkers (2002) sampled the CSF 30 minutes after oral ketoprofen 1 mg/kg from ten children (age 9 months – 7 years) undergoing spinal anaesthesia. They detected ketoprofen in only one CSF sample, because of the insensitive analytical method that had a lower limit of quantification of 20 ηg/ml (Hannu Kokki, personal communication). Mannila et al. (2006) measured ketoprofen concentrations in the CSF after intravenous ketoprofen 1 mg/kg in twenty-one children undergoing spinal anaesthesia. Ketoprofen concentrations were 1.4 -24 ηg/ml, with a rising trend during 7-67 minutes. Ketoprofen concentrations in the CSF and protein-free plasma reached equilibrium at one hour.

CSF concentrations of oxyphenbutazone, pirprofen, diflunisal, rofecoxib, celecoxib, valdecoxib and parecoxib have also been evaluated (Gaucher et al. 1983, Zecca et al. 1988, Nuernberg et al. 1991, Cohen et al. 1998, Buvanendran et al. 2005, Dembo et al. 2005, Mehta et al. 2008). Lipinski's rule of five (Lipinski et al. 2001) suggests that coxib barrier permeation is not poor (molecular weights 314-381 g $mol^{-1}$, logP 3.2-3.9, and up to 1 H-bond donors, and up to 8 H-bond acceptors). For coxibs, plasma protein binding is the major determinant of CNS penetration; celecoxib, rofecoxib and valdecoxib reach 2-3 times higher CSF concentrations than unbound plasma concentrations (Dembo et al. 2005).

**2.8.9 Paracetamol**

The penetration of paracetamol in the CSF has been studied in children, adults and the elderly. Bannwarth and coworkers (1992) administered intravenous propacetamol 2g, corresponding to paracetamol 1g and 14 mg/kg, to 43 adult patients who underwent myelography. Paracetamol concentrations in the CSF ranged between 0.78 and 9.04 mg/l at 20 minutes to 12 hours. The highest concentrations were measured between 45 min and 5 hours. The CSF concentrations exceeded plasma concentrations at 3 hours and later.

Moreau and coworkers (1993) investigated the CSF concentrations of paracetamol in elderly patients with continuous spinal anaesthesia. They detected CSF concentrations ranging between 1.32 and 8.16 mg/l at 15 minutes to 6 hours. The peak occurred at 3 hours after intravenous propacetamol 2g, corresponding to paracetamol 1g.

Anderson et al. (1998) studied the CSF concentrations of oral paracetamol elixir 40 mg/kg in nine ventilator-dependent children aged 5 months – 12 years with external ventricular drains. The typical peak CSF concentration was 20 mg/l at 2.5 hours. Anderson and coworkers estimated a plasma to CSF standardized (to 70 kg) equilibration half-time of 0.72 h (CV 117%) (NONMEM), which is less than that estimated for an effect compartment explaining antipyresis (0.99 h). They suggest that this may indicate that paracetamol requires more time to act on receptors in the CNS. Anderson et al. (1998) compared pharmacokinetic data from children and adults (Bannwarth et al. 1992) and found a longer teq of 2.1 h in adults. However,

their method of using MKMODEL to pooled adult data might have some drawbacks as it loses some between-subject variability. Furthermore, children with head injuries and ventricular drains also had a disrupted BBB (Kawamata et al. 2007, Leonardo and Pennypacker 2009).

Van der Marel and coworkers (2003a) studied paracetamol CSF concentrations in 41 children aged 1 week – 18 years. The children were given rectal paracetamol 32.3 mg/kg before the placement or revision of a ventriculo-peritoneal shunt or the insertion of an external ventricular drain. The median (25-75$^{th}$ percentile) sampling time was 133 min (33 – 202 min) and CSF paracetamol concentrations ranged between 0.0 – 21.0 mg/l. BBB permeation was found to be size- but not age-related and the plasma to CSF equilibration half-time was estimated to be 0.9, 1, 1.4, 1.6 and 1.93 h in a neonate (3.5 kg), 1-year-old child (10 kg), 5-year-old child (20 kg), 10-year-old child (30 kg) and an adult (70 kg), respectively. The standardized (to 70 kg) plasma to CSF teq 1.93 h (CV 43%) was similar to that in adults (2.1 h) (Bannwarth et al. 1992), but higher than in the previous study by Anderson et al (0.72 h) (1998).A possible explanation to this difference is that children in the previous study (Anderson et al. 1998) had head injuries and a disrupted BBB.

Allegaert and coworkers sketched the time course of paracetamol concentrations in the CSF in two neonates with ventricular drains (Allegaert et al. 2004b, Allegaert and Devlieger 2005). In the most recent study, Kozer and coworkers (Kozer et al. 2007) attempted to correlate CSF paracetamol concentrations with antipyretic action in 31 febrile infants aged less than one year. They sampled the CSF 30 minutes to 4 hours after variable doses of oral acetaminophen (mean 14.1 mg/kg), as the mean decrease in body temperature was 1.2ºC. The authors found CSF paracetamol 9.9 ± 5 mg/l, with no differences between boys and girls or between children with and without meningitis. The authors found that body temperature decrement was correlated with time and paracetamol concentrations in the CSF.

Table 9. Main results of previous studies on concentrations of NSAIDs and paracetamol in the CSF

| | **patients** | **dosage** | **sampling times** | **mean Cmax(CSF)** | **tmax (CSF)** |
|---|---|---|---|---|---|
| indomethacin (Bannwarth et al. 1990) | n= 52 24–76y | im 50mg (0.72 kg/mg) | 0.5–12 h | 5 µg/l | 1 h |
| ibuprofen (Bannwarth et al. 1995) | n= 46 25–88y | po 800mg (11.4 mg/kg) | 0.5–8 h | R(-) 168 µg/l S(+) 315 µg/l | 3 h |
| ketorolac (Rice et al. 1993) | n=29 22–71 y | im 90mg (1.2 mg/kg) | 1–4.5 h | 4 µg/l | 2 h |
| paracetamol (Bannwarth et al. 1992) | n=43 31–73 y | iv 1g (14 mg/kg) | 0.3–12 h | 6 mg/l | 2 h |
| paracetamol (Moreau et al. 1993) | n=12 77 ± 7 y | iv 1g (15 mg/kg) | 0.5–6 h | 8 mg/l | 3 h |
| paracetamol (Anderson et al. 1998) | n=9 5 months – 12 y | po 40 mg/kg | 0–10 h | 20 mg/l | 2.5 h |
| paracetamol (van der Marel et al. 2003a) | n=41 1 week – 18 y | rectal 32.3 mg/kg | 0–12 h | 21 mg/l | 2 h |
| paracetamol (Kozer et al. 2007) | n=31 1 week – 9 months | po 8-22 mg/kg | 0.6–4 h | 12 mg/l | 3 h |

# 3 Aims of the study

NSAIDs and paracetamol are analgesic, antipyretic anti-inflammatory drugs commonly used in children. They exert clinically important actions in the CNS. However, CNS penetration of NSAIDs has not been established in children. Therefore, the present study was designed to evaluate CNS penetration of NSAIDs and paracetamol in healthy children. Moreover, an attempt was made to evaluate the analgesic plasma concentrations of ibuprofen and diclofenac in children.

The primary aim of the present study was to:

- evaluate the rate and extent of the CSF penetration of indomethacin (I), ibuprofen (II), ketorolac (III), diclofenac (IV) and paracetamol (V) in healthy children.

The secondary aims were to

- evaluate the effect of demographic parameters on the CSF penetration of non-opioid analgesics (I-V)
- describe the pain relief of ibuprofen (II) and diclofenac (IV) after inguinal surgery with spinal anaesthesia in children.

# 4 Materials and methods

This open-label prospective study was conducted in five consecutive parts (I, II, III, IV, V) at the Kuopio University Hospital in November 2004 – June 2006. The patients were 160 children who were scheduled for elective subumbilical surgery with spinal anaesthesia and were to receive NSAIDs or paracetamol for postoperative pain.

The study protocol was approved by the research ethics committee of the Hospital District of Northern Savo (No. 120/2004). The Finnish National Agency for Medicines was notified (No. 161/2004); the trial was recorded in the EudraCT database (No. 2004-001702-27) and conducted in accordance with the principles of the latest revision of the Declaration of Helsinki (WMA). Written, informed consent was obtained from the parents or legal guardians, and assent from the child.

## 4.1 PATIENTS

The inclusion criteria for the study were 1) age 3 months – 12 years, 2) surgery in the lower part of the body, planned to be performed under spinal anaesthesia, 3) surgery where NSAIDs or paracetamol were planned to be used as preventive pain medication, 4) American Society of Anesthesiologist (ASA) physical status I-II, and 5) informed written consent from parents and assent from the child.

The exclusion criteria for the study were 1) ASA-physical status 3 or above, 2) contraindication to spinal anaesthesia, such as skin infection at the lower back, distinct anatomy of the back, increased intracranial pressure, allergy to local anaesthetics, 3) contraindication to NSAIDs or paracetamol, such as allergy to NSAIDs or paracetamol or any excipients, gastrointestinal ulcer, acetylsalicylic-sensitive asthma, hepatic, renal or cardiac insufficiency, hypovolemia, bleeding disorder, surgery with a significant risk of

haemorrhage, and 4) other reason that the researcher considered to be a contraindication.

Altogether 169 children were found eligible for the study. The parents of 7 children refused consent since they did not want any additional stress for their child. Two children were excluded from the study because their operations were postponed for administrative reasons. Therefore, 160 children were included in the study. Patient baseline characteristics are presented in Tables 10-11.

Table 10. Patient characteristics (range (median) or number of patients)

| | **Age (months)** | **Weight (kg)** | **Height (cm)** | **Gender (boys/ girls)** |
|---|---|---|---|---|
| **Indomethacin (I)** | 4-144 (45) | 7-48 (15) | 63-165 (98) | 23 / 8 |
| **Ibuprofen (II)** | 3-149 (40) | 6-54 (15) | 54-154 (101) | 25/11 |
| **Ketorolac (III)** | 3-134 (46) | 6-49 (15) | 59-159 (100) | 17/13 |
| **Diclofenac (IV)** | 3-153 (56) | 6-60 (21) | 60-169 (111) | 24/7 |
| **Paracetamol (V)** | 3-153 (55) | 7-69 (20) | 60-160 (108) | 19/13 |

Table 11. Surgical operations (number of patients)

| | **Hernio-tomy** | **Orchido-pexy** | **Circum-cision** | **Cysto-scopy** | **Ortho-paedic operation** | **Other** |
|---|---|---|---|---|---|---|
| Indomethacin (I) | 15 | 2 | 2 | 1 | 6 | 5 |
| Ibuprofen (II) | 19 | 1 | 1 | 2 | 6 | 7 |
| Ketorolac (III) | 10 | 4 | - | 2 | 9 | 5 |
| Diclofenac (IV) | 8 | 5 | 4 | 6 | 5 | 3 |
| Paracetamol (IV) | 11 | - | 1 | 10 | 5 | 5 |
| **Total** | **63** | **12** | **8** | **21** | **31** | **25** |

## 4.2 CLINICAL PROTOCOL

*Preoperative care*

Local anaesthetic patches (eutectic mixture of lidocaine and prilocaine, EMLA, Astrazeneca Oy, Espoo, Finland) were placed in the dorsal hands and the back to ease punctures, and an intravenous line was placed preoperatively. In children older than 6 months, a standard buccal premedication was used, with midazolam (0.375 mg/kg up to 7.5 mg, Midazolam Hameln 5 mg/ml, Hameln Pharmaceuticals Gmbh, Hameln, Germany) and ketamine (1.25 mg/kg up to 25 mg, Ketalar 50mg/ml, Pfizer Oy, Espoo, Finland).

*The study drugs*

A body weight-based dose of the study drug was given preoperatively, 5 minutes – 22 hours before anaesthesia. The drugs were given as an intravenous injection over 5-10 minutes with a volumetric infusion pump. The study drugs were:

I: indomethacin 0.35 mg/kg (Confortid 50mg/ml, lot no. 547397-2, exp. date 05-2006, Dumex-Alpharma A/S, Copenhagen, Denmark), diluted in 20 ml normal saline (Natriumklorid Braun 9 mg/ml. B.Braun Melsungen AG, Melsungen, Germany).

II: ibuprofen 10 mg/kg (Ibuprof von ct Amp. 133 mg/ml, lot no. F26775, exp. date 07/2008, ct-Arzneimittel Gmbh, Berlin, Germany), diluted in 20 ml normal saline.

III: ketorolac 0.5 mg/kg (Toradol 30 mg/ml, lot no. B1889, exp. date 10/2006, Roche Oy, Espoo, Finland), diluted in 20 ml normal saline.

IV: diclofenac 1 mg/kg (Voltaren 25 mg/ml, lot no. S0258, exp. date 05/2007, Novartis Finland Oy, Espoo, Finland), diluted in buffered solution. Normal saline 20 ml was buffered with $NaHCO_3$ 7.5% 0.5 ml (Natriumbicarbonate Braun 75 mg/ml, Braun Medical Oy, Helsinki, Finland).

V: paracetamol 15 mg/kg (Perfalgan 10 mg/ml, lot no. 5H00568, exp. date 08/2007, Bristol-Myers Squibb AB, Bromma, Sweden).

*CSF and blood sampling*

One paired CSF and blood sample was collected from each study patient at 5 minutes to 22 hours after drug injection. Lumbar puncture was performed in the lateral decubitus position in the midline at L3-4 with a Quincke G25-27 needle. Cerebrospinal fluid 1 ml was aspirated for a sample, and thereafter local anaesthetic was injected. Within 5 minutes, another intravenous catheter was placed and a blood sample (3 ml) was collected. The intravenous catheter was placed in a different limb from that used for the drug injection.

*Perioperative care*

Before lumbar puncture, the children were sedated with midazolam, thiopental (Pentothal Natrium, Abbott Scandinavia AB, Solna, Sweden) and propofol (Propofol Fresenius Kabi, Fresenius Kabi AB, Uppsala, Sweden). The most commonly used agent for spinal anaesthesia was plain isobaric levobupivacaine 5 mg/ml (Chirocaine®, Abbott Scandinavia AB, Solna, Sweden). The levobupivacaine dose was 0.4 mg/kg in children with body weight less than 16 kg, 0.3 mg/kg in those with body weight 16-40 kg, and 0.25 mg/kg (up to 15 mg) in those with body weight more than 40 kg. Intravenous fluids were given based on clinical needs at the discretion of the attending anaesthetist. The children were given oxygen, $N_2O$ and air via a face mask. During anaesthesia, additional doses of intravenous fentanyl were given when clinically needed at the discretion of the attending anaesthetist.

*Postoperative care*

After surgery, the children were transferred to the post-anaesthesia care unit (PACU). The vital signs, pain and adverse effects (not classified whether drug-related or not) were monitored by the study nurses and the researchers. For postoperative pain, the children received paracetamol intravenously 15 mg/kg and ketoprofen intravenously 1 mg/kg (Orudis 50 mg/ml, Aventis Pharma Oy, Helsinki, Finland), at the discretion of the anaesthetist. For rescue analgesia, children in pain (pain score > 3 at rest and/or >5 on a numeric rating scale of 0-10) were given intravenous fentanyl 1 μg/kg (Fentanyl 50 μg/ml B.Braun Medical oy, Espoo, Finland) or oxycodone 0.05 mg/kg (Oxanest 10mg/ml, Oy Leiras, Helsinki, Finland). Some children, who had undergone a major

operation had continuous epidural opioid - local anaesthetic - epinephrine infusion. Follow-up after discharge from the PACU was arranged according to normal clinical practice.

*Postoperative blood sampling (II, IV)*

Postoperative pain was monitored more closely in a subgroup of children in studies II and IV. Children who had undergone herniotomy with spinal anaesthesia and sedation, and received no other analgesics but the bolus dose of study compounds ibuprofen or diclofenac, were included in the subgroup. Children who had received paracetamol, additional doses of NSAIDs, or opioids, were not included in the group. In study II, the protocol was amended after 31 children were included in the study, and therefore samples were collected from only five children.

Pain was assessed in the PACU by the researchers and trained study nurses every 15 minutes at rest and with a light pressure (20 Newton) on the wound area. Assessment was made by using an 11-point numeric rating scale (0 = no pain, 10 = worst possible pain). The report of the child (when available) and the assessment of the observer were both recorded. When the child first expressed or was assessed to have wound pain, a second blood sample (3 ml) was obtained for the estimation of analgesic plasma concentration of ibuprofen or diclofenac. Thereafter the children received intravenous paracetamol 15 mg/kg and ketoprofen 1 mg/kg, and opioid for rescue analgesics if needed.

### 4.3 DRUG ASSAYS

Indomethacin (I), ibuprofen (II), ketorolac (III) and diclofenac (IV) concentrations in plasma, protein-free plasma and CSF were measured by the gas chromatography-mass spectrometry method. Protein free plasma was obtained by ultra-filtration. Paracetamol (V) concentrations in the plasma and CSF samples were determined by using fluorescence polarization immunoassay technology (TDxFLx; Abbott Laboratories, Abbott Park, Illinois, USA). The methods are described in detail in publications I – V. The accuracy, recovery and intra-day precision are reported in publications I-V.

### 4.4 STATISTICS

No formal sample size calculation was performed, but a sample of 30 children in each group was considered to provide sufficient information on the CSF permeation of each drug. Data were entered and analyzed with the Statistical Package for Social Sciences (SPSS Software versions 11.5, 13.0 and 14.0 for Windows, SPSS Inc., Chicago, USA). The results are presented as number of cases or median with range. The drug concentrations in paired CSF and plasma samples are presented for each individual. The trend line on CSF concentrations versus time plot was generated by applying locally weighted smooth regression (kernel function: Epanechnikov and points to fit: 50%).

In a *post hoc* analysis, the effect of sex, age, height, weight and body surface area (estimated by the DuBois method; DuBois and DuBois 1916) on drug concentrations in the CSF was studied with polynomial regression. First the effect of time was estimated by fitting a cubic regression curve, and then the effect of sex, age, height, weight and body surface area were studied separately with polynomial regression. The predictors were: time, $time^2$, $time^3$, and sex, age, height, weight or body surface area. The dependent variable was drug concentration in the CSF. A p-value of 0.05 was considered as the limit of statistical significance.

### 4.5 CALCULATIONS ON PROTEIN BINDING OF NSAIDS IN THE CSF

Non-protein-bound (free) drug concentrations were measured in plasma, and total drug concentrations were measured in plasma and the CSF. However, protein binding was not determined in the CSF, and therefore calculations on protein binding of NSAIDs in the CSF are presented. The calculations are based on binding equations of NSAID binding to albumin in plasma (Honoré and Brodersen 1984, Borgå and Borgå 1997, Deschamps-Labat et al. 1997, Yamasaki et al. 2000) (Table 12-15). The free drug concentration in the CSF is assumed to be equal to the free drug concentration in plasma, and the median of that is chosen. The CSF albumin concentration is assumed to be $2.3 \times 10^{-6}$ M (Illi et al. 1983), and the molar masses are presented in Table 7.

Table 12. The stoichiometric binding equation by Honoré and Brodersen (Honoré and Brodersen 1984), and the values used in the calculations

$$r_x = \frac{xK_1 + 2x^2K_1K_2 + 3x^3K_1K_2K_3 + \cdots + Nx^NK_1K_2K_3\cdots K_N}{1 + xK_1 + x^2K_1K_2 + x^3K_1K_2K_3 + \cdots + x^NK_1K_2K_3\cdots K_N}$$

rx = number of moles of drug bound per mole of protein
K1, K2…KN = stoichiometric binding constants
x = molar concentration of free drug

| **Indomethacin N=7** | **Ibuprofen N=8** | **Diclofenac N=7** |
|---|---|---|
| $K_1$ = 250 000 | $K_1$ = 80 000 | $K_1$ = 550 000 |
| $K_2$ = 100 000 | $K_2$ = 35 000 | $K_2$ = 190 000 |
| $K_3$ = 55 000 | $K_3$ = 20 000 | $K_3$ = 87 000 |
| $K_4$ = 32 000 | $K_4$ = 13 000 | $K_4$ = 42 000 |
| $K_5$ = 18 000 | $K_5$ = 8 000 | $K_5$ = 22 000 |
| $K_6$ = 9 500 | $K_6$ = 5 500 | $K_6$ = 11 500 |
| $K_7$ = 3 900 | $K_7$ = 2 900 | $K_7$ = 4 300 |
| x = 1.3966 x $10^{-9}$ | $K_8$ = 1 300 | x = 2.7027 x $10^{-9}$ |
| | x = 0.3495 x$10^{-6}$ | |

Table 13. Model by Borgå and Borgå (Borgå and Borgå 1997) with high and low affinity binding sites, and the values used in the calculations

$$\frac{C_b}{Alb} = \frac{N1 \times C_u}{K1 + C_u} + \frac{N2 \times C_u}{K2 + C_u}$$

Cb = concentration of bound drug
Alb = albumin concentration
$C_u$ = concentration of free drug
N1, N2= number of binding sites for high and low affinity sites
K1, K2 = dissociation constants for high and low affinity binding sites

**Diclofenac**
N1= 1.80
K1 = 2.57 x $10^{-6}$
K2 = 8.50
K2= 108 x $10^{-6}$
Alb = 2.3 x $10^{-6}$
$C_u$ = 2.7027 x $10^{-9}$

Table 14. Model by Deschamps-Labat and coworkers with two saturable binding sites (Deschamps-Labat et al. 1997), and the values used in the calculations

| | |
|---|---|
| $r = \frac{n_1 K_1 F}{1 + K_1 F} + \frac{n_2 K_2 F}{1 + K_2 F}$<br><br>r = number of moles of drug bound per mole of protein<br>$n_1$ = number of binding sites per mole of albumin for site I<br>$K_1$ = association constant for site I<br>$n_2$ = number of binding sites per mole of albumin for site II<br>$K_2$ = association constant for site II<br>F = molar concentration of free drug | **Ibuprofen**<br>$n_1$=0.98<br>$K_1$=434.56 x $10^3$<br>$n_2$= 5.12<br>$K_2$= 8.59 x $10^3$<br>F=0.3495 x$10^{-6}$ |

Table 15. Model by Yamasaki et al. (Yamasaki et al. 2000) with two binding sites, high and low affinity, and the values used in the calculations

| | |
|---|---|
| $r = \frac{n_h K_h C_f}{1 + K_h C_f} + \frac{n_l K_l C_f}{1 + K_l C_f}$<br><br>r = number of moles of drug bound per mole of protein<br>$K_h$, $K_l$ = association constants for high and low affinity binding sites<br>$n_h$, $n_l$ = number of binding sites for high and low affinity binding sites<br>$C_f$ = molar concentration of free drug | |
| **Ibuprofen**<br>$K_h$ = 3.3 x $10^6$<br>$K_l$ = 5.5 x $10^4$<br>$n_h$ = 1.0<br>$n_l$ = 4.8<br>$C_f$ = 0.3495 x$10^{-6}$ | **Diclofenac**<br>$K_h$ = 3.3 x $10^6$<br>$K_l$ = 5.4 x $10^4$<br>$n_h$ = 1.0<br>$n_l$ = 4.9<br>$C_f$ = 2.7027 x$10^{-9}$ |

### 4.6 PROTOCOL DEVIATIONS

There were five major protocol deviations in the study drug administration. In study III, one child received ketorolac 0.35 mg/kg subcutaneously, instead of 0.5 mg/kg intravenously, due to extravasation of the injection. In study V, one child was given paracetamol 20 mg/kg and one 12.5 mg/kg instead of 15 mg/kg, and two children had paracetamol infusion over 30 and 40 minutes instead of 5-10 minutes. These cases were excluded.

There were 17 deviations in the CSF samples (Table 16). Four CSF samples were reddish, indicating blood contamination; these were excluded. Thirteen CSF samples had the drug concentrations below the limit of quantitation; for statistical analysis they were replaced with the value calculated as limit of quantitation/2, as indicated in Figures 12-16. The lower limit of quantification of indomethacin, ibuprofen, ketorolac, diclofenac and paracetamol assay was 0.1 μg/l, 4.0 μg/l, 0.1 μg/l, 0.1 μg/l and 1.0 mg/l, respectively.

Table 16. Protocol deviations in the study

| | **Major deviation in the study drug administration** | **CSF sample was reddish** | **CSF samples with drug concentration below the limit of quantitation** |
|---|---|---|---|
| Indomethacin (I) | 0 | 2 | 2 |
| Ibuprofen (II) | 0 | 0 | 0 |
| Ketorolac (III) | 1 | 1 | 8 |
| Diclofenac (IV) | 0 | 0 | 2 |
| Paracetamol (V) | 4 | 1 | 1 |
| **Total** | **5** | **4** | **13** |

# 5 RESULTS

## 5.1 DRUG CONCENTRATIONS IN THE CSF

The range and median of drug concentrations in the CSF, plasma, and protein-free plasma, and the ratios of concentrations in those three compartments are shown in Table 17 and Figures 12-16.

Table 17. Summary of the drug concentrations in the CSF and plasma

| | **Indomethacin (I)** | **Ibuprofen (II)** | **Ketorolac (III)** | **Diclofenac (IV)** | **Paracetamol (V)** |
|---|---|---|---|---|---|
| **Total CSF concentration (µg/l)** | n=27 | n=36 | n=20 | n=29 | n=26 |
| **range** | 0.2-5.0 | 15-541 | 0.2-3.0 | 0.1-4.7 | 1.3-18 x $10^3$ |
| **median** | 1.4 | 182 | 0.5 | 1.2 | 7.2 x $10^3$ |
| **Free plasma concentration (µg/l)** | n=20 | n=36 | n=29 | n=22 | |
| **range** | 0.3-0.9 | 4.8-604 | 2.0-31.9 | 0.4-3.8 | not studied |
| **median** | 0.5 | 72 | 9.1 | 0.8 | |
| **Total plasma concentration (µg/l)** | n=31 | n=36 | n=29 | n=29 | n=28 |
| **range** | 91-2233 | 1475-89268 | 449-4831 | 55-4232 | 2.4-33 x $10^3$ |
| **median** | 776 | 43317 | 2603 | 540 | 15 x $10^3$ |
| **Protein binding in plasma (%)** | | | | | |
| **range** | 99.92-99.97 | 99.32-99.92 | 99.18-99.87 | 99.40-99.95 | not studied |
| **median** | 99.96 | 99.80 | 99.66 | 99.90 | |
| **Ratio CSF to free plasma** | | | | | |
| **range** | 0.4-15 | 0.42-8.6 | 0.014-0.36 | 0.2-5.2 | not studied |
| **median** | 3.1 | 2.3 | 0.08 | 2.2 | |
| **Ratio CSF to total plasma** | | | | | |
| **range** | 0.0001-0.01 | 0.0015-0.021 | 0.00007-0.0015 | 0.0002-0.05 | 0.06-2 |
| **median** | 0.002 | 0.0048 | 0.00021 | 0.005 | 0.8 |

*Indomethacin (I)*

Indomethacin was detected in 27 out of 29 CSF samples collected at 14 minutes to 3.75 hours after indomethacin 0.35 mg/kg iv. Two samples collected at 14 and 19 minutes had indomethacin below the limit of quantitation 0.1 µg/l. Indomethacin concentrations in 27 CSF samples ranged between 0.2 and 5.0 µg/l, with a median of 1.4 µg/l, and the highest concentrations were detected at 1 hour (Figure 12).

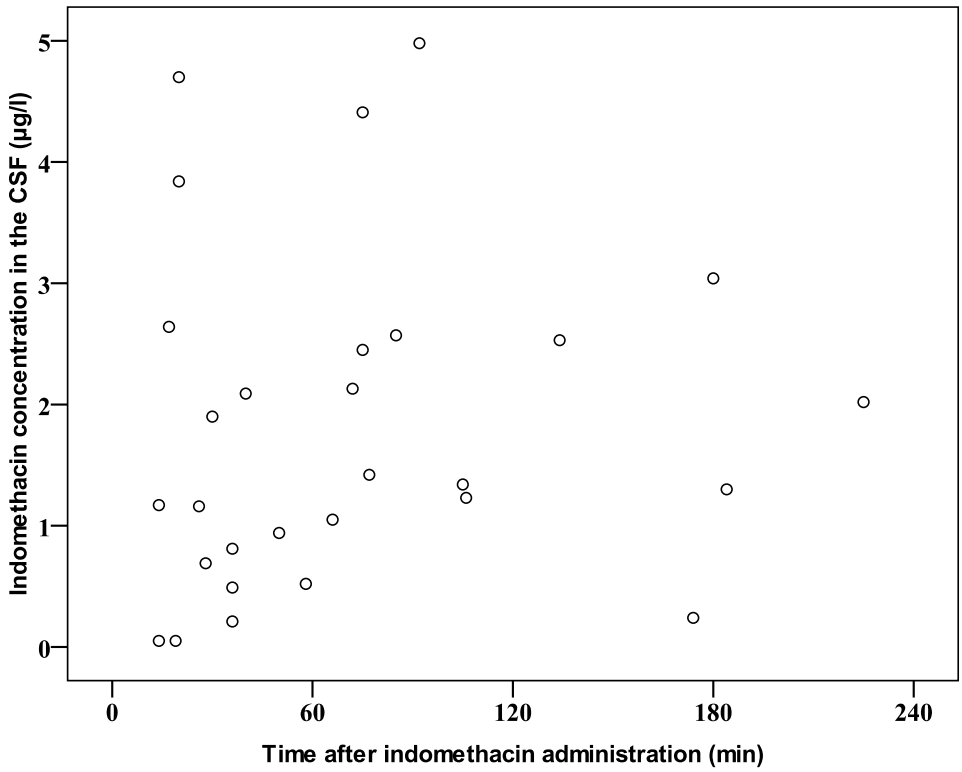


Figure 12. Indomethacin concentrations in the CSF in 29 children after indomethacin 0.35 mg/kg iv

*Ibuprofen (II)*

Ibuprofen was detected in all 36 CSF samples collected at 10 minutes to 8 hours after ibuprofen 10 mg/kg iv. Ibuprofen concentrations in the CSF ranged between 15 and 541 µg/l (median 182 µg/l) and the highest concentrations were detected at 1 hour (Figure 13).

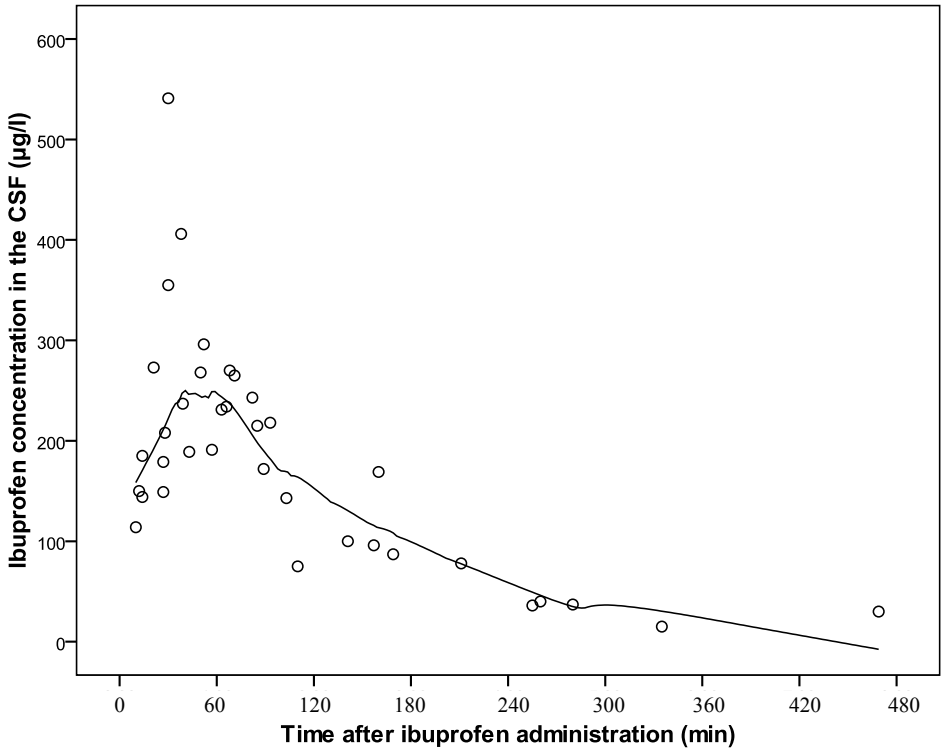


Figure 13. Ibuprofen concentrations in the CSF in 36 children after ibuprofen 10 mg/kg iv and locally weighted smooth regression trend line

*Ketorolac (III)*

Ketorolac was detected in 20 out of 28 CSF samples collected at 13 minutes to 5.5 hours after ketorolac 0.5 mg/kg iv. Ketorolac concentrations were below the limit of quantitation, 0.1 µg/l, in eight samples collected at 11-19 minutes and at 1-6 hours after the drug administration. Ketorolac concentrations in the CSF ranged between 0.2 and 3.0 µg/l (median 0.5 µg/l), and the highest concentrations were detected at 1 hour (Figure 14).

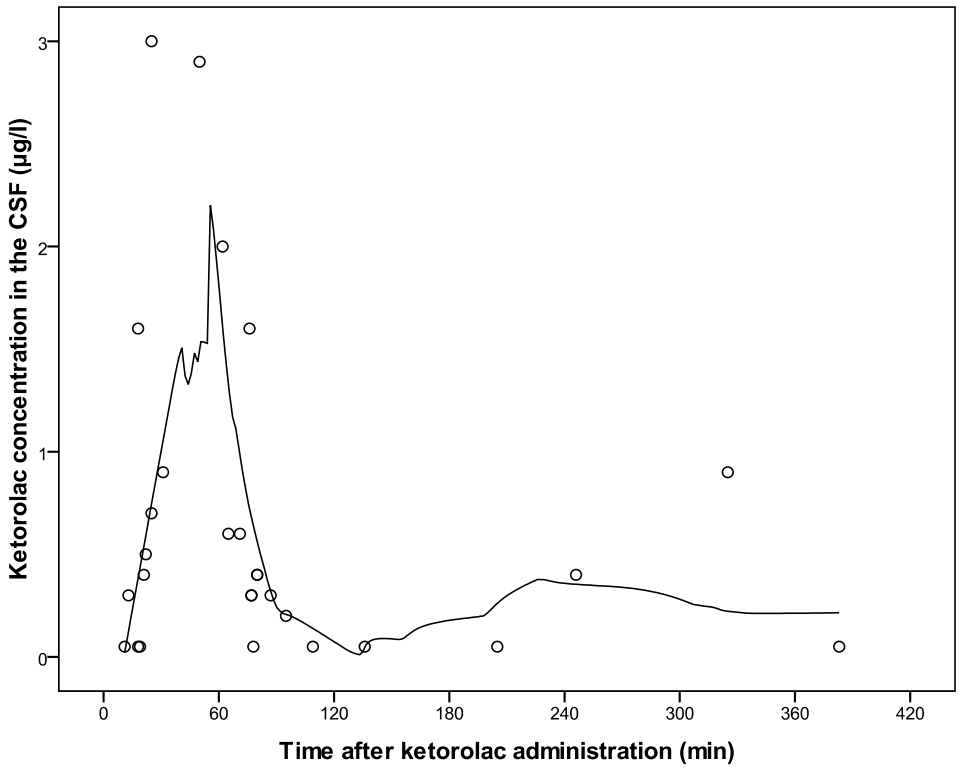


Figure 14. Ketorolac concentrations in the CSF in 28 children after ketorolac 0.5 mg/kg iv and locally weighted smooth regression trend line

*Diclofenac (IV)*

Diclofenac was detected in 29 out of 31 CSF samples at 5 minutes to 5.5 hours after diclofenac 1 mg/kg iv. Diclofenac concentration in the CSF was below the limit of quantitation 0.1 µg/l in two samples collected at 22 hours. The concentrations in the CSF were 0.1–4.7 µg/l (median 1.2 µg/l), and the highest concentrations were detected at 1 hour (Figure 15).

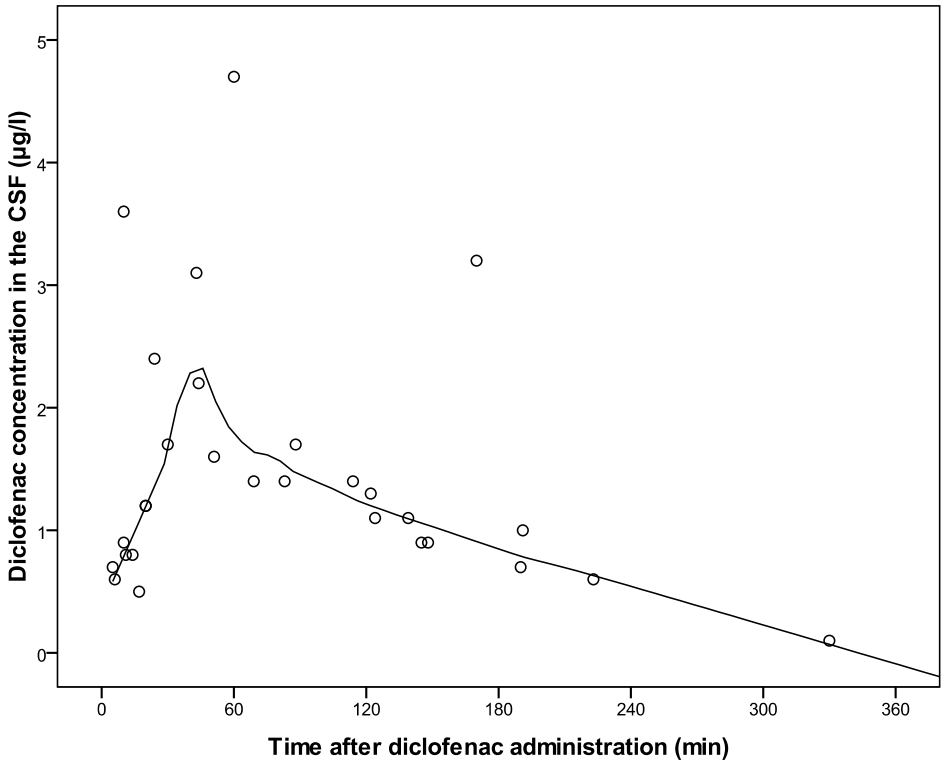


Figure 15. Diclofenac concentrations in the CSF in 29 children after diclofenac 1 mg/kg iv and locally weighted smooth regression trend line

*Paracetamol (V)*

Paracetamol was detected in 26 out of 27 CSF samples collected at 6 minutes to 5 hours after paracetamol 15 mg/kg iv. One sample at 5 minutes had a paracetamol concentration below the limit of quantitation of 1.0 mg/l. Paracetamol concentrations in the CSF ranged between 1.3 and 18.0 mg/l with the median of 7.2 mg/l, and the highest concentrations were detected at 1-2 hours (Figure 16).

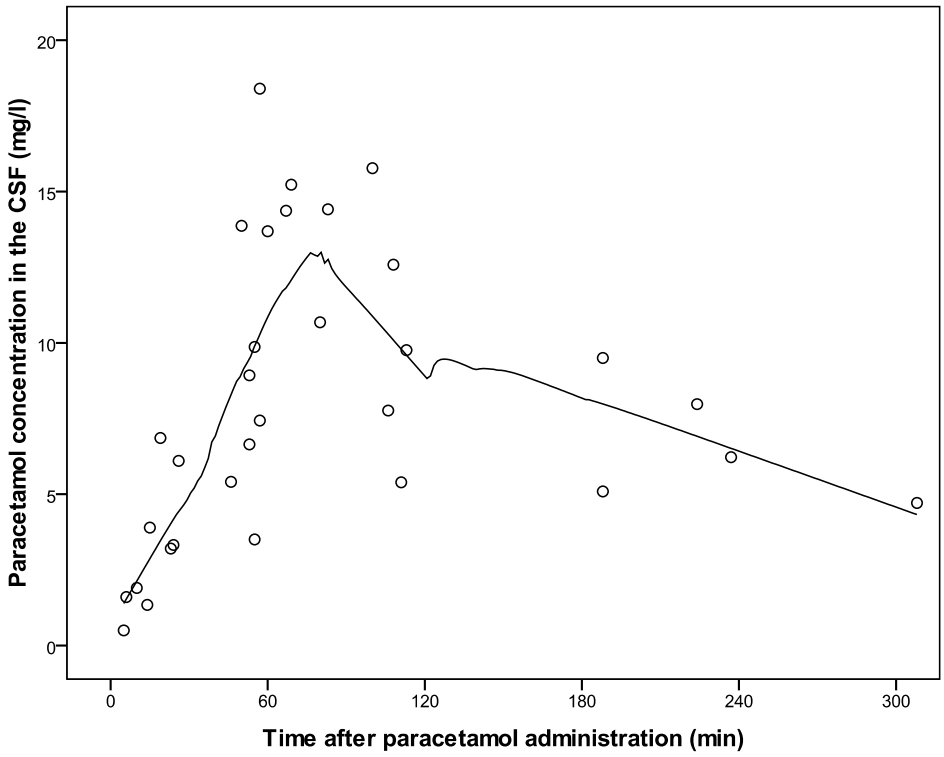


Figure 16. Paracetamol concentrations in the CSF in 27 children after paracetamol 15 mg/kg iv and locally weighted smooth regression trend line

## 5.2 DRUG CONCENTRATIONS IN THE CSF AND PATIENT CHARACTERISTICS

The polynomial regression curves fit to the data on ibuprofen, diclofenac and paracetamol at the time ranges of 10-469, 5-333 and 5-308 minutes, respectively (Figure 17). For indomethacin, the effect of time was estimated by fitting a linear curve (Figure 17). An attempt to fit a curve to the data on ketorolac was unsuccessful, and therefore analyses were not performed. Indomethacin and diclofenac concentrations in the CSF were related to age, height, weight and body surface area of the children. Younger and smaller children had higher drug concentrations in the CSF than did older and taller children. Ibuprofen concentrations in the CSF were not related to sex, age, height, weight or body surface area. Paracetamol CSF concentrations were related to sex: girls had higher concentrations of paracetamol in the CSF than boys. The p-values are shown in Table 18.

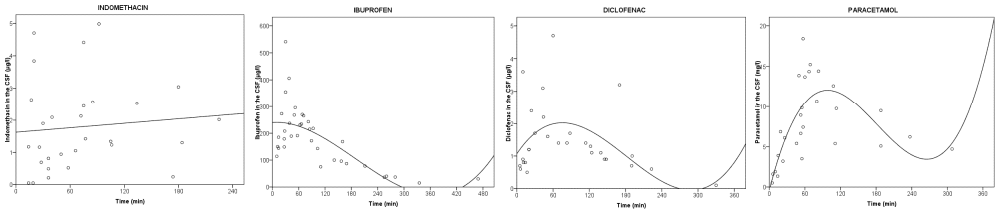


Figure 17. The polynomial regression curves for data on indomethacin, ibuprofen, diclofenac and paracetamol, respectively

Table 18. The relation of drug CSF concentrations and patient characteristics. P-values of the regression analyses

| | **sex** | **age** | **height** | **weight** | **body surface area** |
|---|---|---|---|---|---|
| **indomethacin**[a] | ns | 0.006 | 0.01 | 0.041 | 0.019 |
| **ibuprofen** | ns | ns | ns | ns | ns |
| **diclofenac** | ns | 0.014 | 0.002 | 0.033 | 0.010 |
| **ketorolac**[b] | | | | | |
| **paracetamol** | 0.009 | ns | ns | ns | ns |

a. the effect of time was estimated with a linear instead of a cubic curve
b. no analyses, because the cubic curve did not fit the data

### 5.3 KETOROLAC CONCENTRATIONS IN PLASMA SAMPLES (III)

Ketorolac concentrations in plasma ranged between 449 and 4831 μg/l (2603 μg/l). Protein free plasma concentrations ranged between 2.0 and 31.9 μg/l (9.1 μg/l). Ketorolac concentrations in the CSF, protein free plasma (unbound) and total plasma in each patient are shown in Table 19. This table contains the correct data. In original publication III, the data in the last column of Table I are incorrect.

Table 19. Cerebrospinal fluid (CSF), unbound plasma and total plasma ketorolac concentrations in each patient (III)

| Patient number | Gender | Age (months) | Height (cm) | Weight (kg) | Sampling time (min) | CSF concentration (µg/l) | Unbound plasma concentration (µg/l) | Total plasma concentration (µg/l) |
|---|---|---|---|---|---|---|---|---|
| 24* | male | 61 | 121 | 22 | 11 | ND | 14.9 | 3093 |
| 17 | female | 47 | 103 | 17 | 13 | 0.3 | 15.6 | 3521 |
| 22 | female | 16 | 80 | 11 | 18 | 1.6 | 15.4 | 4386 |
| 29 | female | 122 | 141 | 33 | 18 | ND | 13.9 | 2431 |
| 3 | male | 25 | 96 | 18 | 19 | ND | 31.9 | 4496 |
| 13 | male | 24 | 89 | 12 | 21 | 0.4 | 29.7 | 3617 |
| 21 | male | 26 | 93 | 15 | 22 | 0.5 | 12.1 | 3953 |
| 23 | male | 13 | 76 | 10 | 25 | 0.7 | 8.3 | 4352 |
| 30 | male | 10 | 75 | 11 | 25 | 3.0 | 25.8 | 4831 |
| 26 | male | 6 | 67 | 8 | 31 | 0.9 | 7.7 | 2618 |
| 19 | male | 3 | 59 | 6 | 32 | 7.6a | 11.0 | 3245 |
| 14* | male | 31 | 85 | 13 | 50 | 0.2b | 5.5b | 1553b |
| 20 | male | 6 | 65 | 6 | 50 | 2.9 | 9.8 | 3851 |
| 4* | female | 55 | 110 | 18 | 62 | 2.0 | 10.8 | 3889 |
| 11* | female | 11 | 76 | 12 | 65 | 0.6 | 3.3 | 2623 |
| 25* | male | 114 | 143 | 35 | 71 | 0.6 | 13.7 | 1738 |
| 27 | male | 37 | 94 | 13 | 76 | 1.6 | 9.1 | 1985 |
| 1 | female | 67 | 120 | 20 | 77 | 0.3 | 4.1 | 2160 |
| 28 | male | 80 | 123 | 24 | 77 | 0.3 | 9.1 | 1463 |
| 5* | male | 70 | 115 | 22 | 78 | ND | 6.9 | 1265 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 10 | male | 46 | 101 | 15 | 80 | 0.4 | 7.5 | 2622 |
| 12 | female | 53 | 98 | 14 | 80 | 0.4 | 11.2 | 2603 |
| 8 | female | 113 | 136 | 29 | 87 | 0.3 | 5.0 | 1680 |
| 15 | female | 88 | 119 | 24 | 95 | 0.2 | 3.8 | 1922 |
| 6* | male | 74 | 120 | 24 | 109 | ND | 4.3 | 1522 |
| 7 | female | 54 | 110 | 15 | 136 | ND | 5.8 | 1883 |
| 9 | female | 134 | 159 | 49 | 205 | ND | 3.3 | 1560 |
| 2 | male | 35 | 94 | 13 | 246 | 0.4 | 2.0 | 579 |
| 16 | female | 4 | 63 | 7 | 325 | 0.9 | 2.6 | 623 |
| 18 | female | 122 | 138 | 33 | 383 | ND | 2.0 | 449 |
| **minimum** | | 3 | 59 | 6 | 11 | 0.2 | 2.0 | 449 |
| **maximum** | | 134 | 159 | 49 | 383 | 7.6 | 31.9 | 4831 |
| **median** | | 47 | 101 | 15 | 71 | 0.6 | 9.1 | 2603 |



* CNS adverse effect.

ND none detected, concentration was below the lower limit of quantification of 0.1 μg/l.

a. blood-stained sample.

b. received ketorolac 0.35 mg/kg subcutaneously.

## 5.4 CALCULATIONS ON PROTEIN BINDING IN THE CSF

The models by Honoré and Brodersen (1984), Borgå and Borgå (1997), Deschamps-Labat et al. (1997) and Yamasaki et al. (2000) were applied. The results of calculations are presented in Table 20. According to the models, indomethacin was 37%, ibuprofen 15-49%, and diclofenac 56-89% bound to albumin in the CSF.

Table 20. Calculations of protein binding of NSAIDs in the CSF

| | Assumed free drug in the CSF (µg/l) | Calculated total drug in the CSF (µg/l) | Calculated bound drug in the CSF | Reference |
|---|---|---|---|---|
| Indomethacin | 0.5 | 0.78 | 37% | (Honoré and Brodersen 1984) |
| Ibuprofen | 72 | 140 | 49% | (Deschamps-Labat et al. 1997) |
| Ibuprofen | 72 | 85 | 15% | (Honoré and Brodersen 1984) |
| Ibuprofen | 72 | 125 | 43% | (Yamasaki et al. 2000) |
| Diclofenac | 0.8 | 1.8 | 56% | (Honoré and Brodersen 1984) |
| Diclofenac | 0.8 | 2.2 | 64% | (Borgå and Borgå 1997) |
| Diclofenac | 0.8 | 7.3 | 89% | (Yamasaki et al. 2000) |

## 5.5 PLASMA CONCENTRATIONS AT ONSET OF PAIN AFTER SURGERY

*Ibuprofen (II)*

Three samples were collected as defined in the protocol (Table 21). Pain occurred at 266, 186, 222 minutes after ibuprofen administration, at 97, 129, 208 minutes after spinal anaesthesia and at 57, 116, 171 minutes after the end of surgery. The pain intensity in NRS (0-10, at rest/with light (20N) pressure on the wound) was assessed to be 1/3, 1/4 and 2/3. Ibuprofen concentrations in plasma and protein free plasma at the onset of pain were 10 $x10^3$, 25 $x10^3$, 11 $x10^3$ μg/l and 15, 26, 32 μg/l, respectively (Figure 18).

Table 21. Ibuprofen concentrations in plasma at onset of pain after surgery

| | | onset of pain after (min) | | | ibuprofen concentration (μg/l) | |
|---|---|---|---|---|---|---|
| **patient number** | **operation** | **ibuprof inj** | **spinal anaesth** | **surgery** | **plasma** | **free plasma** |
| 33 * | herniotomy | 213 | 201 | 169 | 21 $x10^3$ | 20 |
| 34 | herniotomy | 266 | 97 | 57 | 10 $x10^3$ | 15 |
| 35 ** | herniotomy | 189 | 175 | 138 | 25 $x10^3$ | 157 |
| 36 | herniotomy | 186 | 129 | 106 | 25 $x10^3$ | 26 |
| 37 | herniotomy | 222 | 208 | 171 | 11 $x10^3$ | 32 |

* The sample was collected 50 minutes after pain occurrence, and was therefore excluded

** The patient had received fentanyl 10 μg after surgery, and was therefore excluded

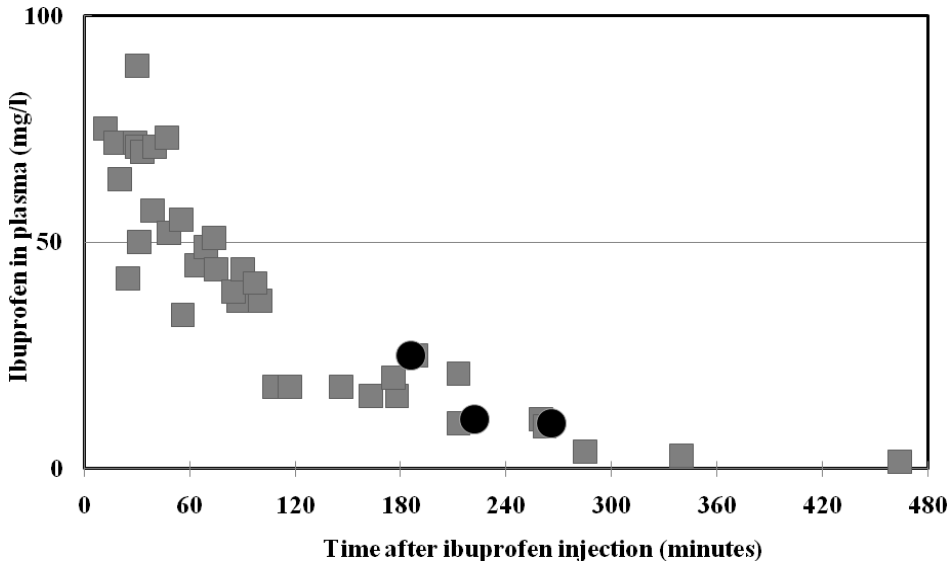


Figure 18. Ibuprofen concentrations in plasma (x $10^3$ μg/l). Black circles represent samples collected at onset of pain after inguinal surgery and spinal anaesthesia. Grey squares represent other plasma samples.

*Diclofenac (IV)*

Eight samples were collected as defined in the protocol (Table 22). Pain occurred at 97-316 (183) minutes after diclofenac administration, at 91-296 (134) minutes after spinal anaesthesia and at 20-272 (98) minutes after the end of surgery. Pain intensity at the onset of pain in NRS was assessed to be 0-3/10 at rest and 2-6/10 with light (20N) pressure on the wound. Diclofenac concentrations in plasma in seven samples ranged between 70 and 272 (median 104) μg/l, and diclofenac was not detected in one sample (Figure 19).

Table 22. Diclofenac concentrations in plasma at onset of pain after surgery

| | | **onset of pain after (min)** | | | **ibuprofen concentration (μg/l)** | |
|---|---|---|---|---|---|---|
| **patient number** | **operation** | **ibuprof inj** | **spinal anaesth** | **surgery** | **plasma** | **free plasma** |
| 1 | orchidopexy | 194 | 180 | 106 | 102 | ND |
| 3 * | circumcision | 322 | 200 | 175 | ND | ND |
| 7 * | orthopaedic | 472 | 142 | 87 | ND | ND |
| 12 * | orthopaedic | 272 | 203 | 169 | 75 | ND |
| 15 * | orthopaedic | 408 | 217 | 159 | ND | ND |
| 16 * | orthopecid | 240 | 180 | 145 | 133 | 0.4 |
| 17 | herniotomy | 295 | 125 | 94 | ND | ND |
| 19 | herniotomy | 108 | 98 | 50 | 272 | 0.6 |
| 25 | orchidopexy | 316 | 296 | 271 | 104 | 0.7 |
| 28 | herniotomy | 97 | 91 | 20 | 163 | ND |
| 29 | herniotomy | 142 | 112 | 77 | 124 | ND |
| 30 | herniotomy | 160 | 143 | 102 | 94 | ND |
| 31 | orchidopexy | 130 | 225 | 163 | 70 | ND |

* The operation was not herniotomy or orhciopexy, and was therefore excluded

ND none detected, concentration was below the lower limit of quantification (58 μg/l for plasma and 0.1 μg/l for protein-free plasma)

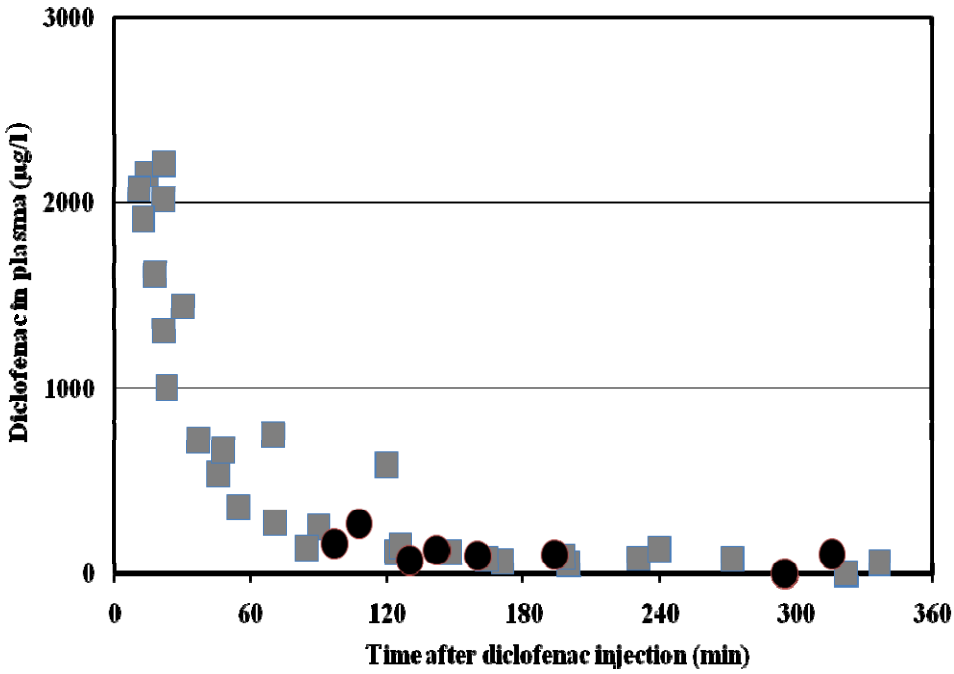


Figure 19. Diclofenac concentrations in plasma (µg/l). Black circles represent samples collected at onset of pain after inguinal surgery and spinal anaesthesia. Grey squares represent other plasma samples. One sample at 13 minutes with concentration 4232 µg/l, and two samples at 22 hours with concentration below the limit of quantification are not shown.

### 5.6 THE ADVERSE EFFECTS

A total of 41 adverse effects occurred in 40 out of 160 patients (Table 23). CNS adverse effects, agitation and anxiety, were reported in 19 children. Gastrointestinal adverse effects, nausea, vomiting and abdominal pain, occurred in 16 children. No serious or unexpected adverse effects occurred. The likelihood of the study drugs causing the reaction was not rated.

Table 23. Reported adverse effects (number of patients)

| | Indomethacin (n=31) | Ibuprofen (n=36) | Ketorolac (n=30) | Diclofenac (n=31) | Paracetamol (n=32) | total (n=160) |
|---|---|---|---|---|---|---|
| **CNS** | | | | | | |
| **agitation/ anxiety** | 5 | 3 | 7 | 1 | 3 | 19 |
| **GI** | | | | | | |
| **vomiting** | 3 | 1 | 1 | 2 | 1 | 8 |
| **nausea** | 1 | 2 | | 2 | 1 | 6 |
| **abdominal pain** | 2 | | | | | 2 |
| **shivering** | 1 | | | 2 | 1 | 4 |
| **urticaria** | | | 1 | | | 1 |
| **coughing** | | 1 | | | | 1 |
| **Total** | 12 | 7 | 9 | 7 | 6 | 41 |

# 6 Discussion

## 6.1 GENERAL DISCUSSION

### 6.1.1 Study population

NSAID permeation in the CNS has previously been studied in adults (Bannwarth et al. 1990, 1992, 1995, Rice et al. 1993) but there are few studies in children. Paracetamol concentrations in the CSF have been studied in children with a disturbed BBB (Anderson et al. 1998, van der Marel et al. 2003a), and ketoprofen concentrations in the CSF in healthy children (Kokki et al. 2002, Mannila et al. 2006). Therefore, the present study evaluated the CSF concentrations of various NSAIDs and paracetamol in healthy children. CSF samples were collected during lumbar puncture for spinal anaesthesia. None of the patients had head trauma, CNS infections or other diseases of the CNS that may affect the BBB permeation. The children had elective surgery, and they were not suffering from major trauma or systemic inflammatory or infectious process. Therefore, it seems that the patients had a non-disturbed BBB, and thus the results should be soundly based.

### 6.1.2 Study design

In the present study, intravenous dosing of NSAIDs and paracetamol was used to eliminate the effect of absorption and first-pass metabolism. However, only one CSF sample was collected per study patient. Samples were collected at induction of spinal anaesthesia, so it was possible to collect only one CSF sample per patient. Multiple samples at different time points would have given more information about the CNS kinetics of NSAIDs and paracetamol in children.

There are some important limitations when CSF concentrations are used to evaluate target concentrations in the brain (de Lange and Danhof 2002). Brains consist of multiple compartments (Figure 7), and many factors affect the transport of drugs between the compartments. Different drugs have different

effect compartments in the brain, such as the intracellular fluid (for anti-HIV drugs, cytostatics, NSAIDs) and the extracellular fluid (for antibiotics). Biological barriers such as the ependyma and the BBB are active and metabolic barriers, which limit the transport of drugs between different compartments; therefore, concentrations measured in one compartment do not necessarily correlate with those in other compartments. Furthermore, the measured lumbar CSF drug concentrations may differ from brain ventricular CSF concentrations. Moreover, some regional differences may occur as a result of physiological and pathological conditions. It seems that lumbar CSF drug concentrations (measured in the present study) are similar to thoracic CSF drug concentrations, because of the constant flow of the CSF and the short distance involved. In addition, the spinal targets of NSAIDs are in close contact with thoracic CSF (Svensson and Yaksh 2002), and therefore lumbar CSF concentrations can be considered relevant for the spinal action of NSAIDs.

### 6.1.3 Analytical methods

In the present study, NSAID concentrations in the CSF were measured by a highly selective and sensitive gas chromatography-mass spectrometry method. Previously the sensitivity of the assay has been the limiting factor when NSAID CSF permeation has been studied in children (Kokki et al. 2002, Mannila et al. 2006). The current method was highly sensitive, and we were able to quantify the concentrations of NSAIDs in 113 out of 125 CSF samples (in I-IV). The validity of the method was partially studied according to FDA guideline for bioanalytical method validation (FDA 2001).

The precision of the indomethacin assay (I) was good for indomethacin concentrations 0.8 – 15 μg/l (CV 3-15%), but poorer for concentrations in the lower range of the method (0.1 μg/l) (CV 34%). Indomethacin concentrations in plasma were above 90 μg/l, and in the CSF mostly above 0.8 μg/l (with five samples below this). Therefore the results of indomethacin in plasma and the CSF can be considered precise. However, indomethacin protein free plasma concentrations were in the lower range of the method, ranging between 0.3 and 0.8 μg/l. Therefore the values of protein free plasma indomethacin should be interpreted with caution. However, despite problems with the precision,

the accuracy was good (98 – 122%) also in the lower range of the method The precision of the other assays used, was good (CV <20%).

In the present study, the NSAIDs ketorolac and ibuprofen were administered as racemic drugs, which are used in normal clinical practice. It seems that only the S-forms of ibuprofen and ketorolac have analgesic actions (Malmberg and Yaksh 1992, Mroszczak et al. 1996, Yaksh et al. 2001), so it would have been interesting to measure the concentrations of S- and R-isomers in the CSF. Such analysis would have been possible, but the sensitivity would have been considerable poorer (100-1000 times lower, due the use of different ionization method). Poorer sensitivity would have been a problem especially with ketorolac, because ketorolac concentrations in the CSF were low and the amount of CSF available for analysis was limited. Therefore the concentrations of racemic drugs, instead of both isomers, were analyzed.

### 6.1.4 Statistical methods

Polynomial regression analysis was used to evaluate the effect of demographic parameters on the CSF penetration of the drugs. This method has many limitations in the situation and it could not be used for the data on ketorolac. Pharmacokinetic methods, non-linear regression or the use of smoothed splines would have been better for this evaluation. However, this study was not designed to evaluate the effect of demographic parameters, and a different study design with stratification or randomization would have improved such an evaluation.

### 6.1.5 Ethical aspects

The importance of paediatric studies in pharmacology has been noted in recent years (Caldwell et al. 2004). The World Health Organization (WHO) launched an initiative in 2007 to make drugs available for children. A new paediatric regulation entered into force on 26$^{th}$ January 2007 in the European Union, requiring new drugs to be studied in children, and offers extended patents for old drugs if they are studied in children (The European Parliament and the Council 2006). Furthermore, the paediatric subpopulation is discussed in document E11 by ICH (International conference on harmonisation of technical requirements for registration of pharmaceuticals for human use)

(ICH 2000). In the United States of America, the act Best Pharmaceuticals for Children promotes paediatric clinical trials (107th Congress of the United States of America 2002).

*Consent*

A paediatric population represents an especially vulnerable subgroup. Children are not legally able to provide informed consent for study participation, and therefore consent is sought from the parents or legal representatives on the child's behalf. However, children older than 15 years are considered mature enough to give consent to a study that is beneficial to them, but their legal guardians are also notified. Moreover, children at all ages should receive information about the study, according to their capacity to understand. Children's opinions on study participation should be respected and therefore written or non-written assent should be obtained whenever a child is able to give it. Recently, after this study was conducted, new recommendations have been published on paediatric studies and on the information to be provided to children (Finpedmed).

In this study, informed written consent was obtained from parents or legal representatives of the child. The parents were given explanations about the normal anaesthesia method and the study interventions, and the aims of the study. They were also provided written information about the study (approved by the ethics committee), and given the opportunity to ask questions and time to consider participation and to talk with their spouse. If the child presented in the hospital the day before surgery, recruitment was preformed then. If the child had day-case surgery and presented only in the morning, the parent was contacted by phone in the previous afternoon and the study protocol was briefly explained, thereby allowing time to consider participation; and written consent was asked for in the morning. If the child was considered old enough to understand the study interventions, information was provided to him/her too and written assent was obtained.

*Study interventions*

It is considered unethical to recruit healthy volunteers for studies on paediatric pharmacology. Furthermore, performing invasive procedures, such

as lumbar puncture, involves risks (such as post-dural punction headache, back pain, neurological symptoms, infection and subdural haematoma). In this study CSF was collected during lumbar puncture, which was indicated for spinal anaesthesia and surgery. The volume of CSF sample was 1 ml or less, therefore smaller than the volume of intrathecally injected local anaesthetic. Therefore, CSF sampling should have had minimal additional risks. Moreover, analyses on data collected on spinal anaesthesia in children indicate that CSF aspiration does not affect the spread and duration of sensory block, or the recovery from spinal anaesthesia in children (Hannu Kokki, unpublished observation). Numerous studies indicate that spinal anaesthesia is a feasible method also in children (Kokki and Hendolin 1995, Kokki et al. 2000, Kokki et al. 2004).

In paediatric clinical research, additional pain and distress, such as venepuncture for blood samples, should be avoided (ICH 2000, Lötjönen et al. 2008). In normal practice at the Kuopio University Hospital, an intravenous line is placed in healthy children coming to elective surgery in the operation room at anaesthesia induction. Parents are not allowed in the operation room, since parents have little impact on the performance of a premedicated child at induction (Kain et al. 2006, Chundamala et al. 2009, Yip et al. 2009). In this study, an intravenous line was placed on the surgical ward or the day-case surgery centre playroom, in the presence of the parents. This allowed the parents to support their child, as needle insertion causes anxiety and sometimes hurts despite the local anaesthetic patch (Cordoni and Cordoni 2001). The second intravenous catheter was placed in sedated children under spinal anaesthesia, most commonly in a dorsal foot vein, after the onset of sensory block by spinal anaesthesia, and therefore should not have caused any pain or distress to the children.

*Pain control*

Paediatric postoperative pain management studies are sometimes considered unethical (Korpela et al. 1999, Black and Mackersie 2000). In the subpopulation of this study, children (n=5 in study II and n=13 in study IV) were provided with one bolus dose of preventive pain medication and spinal anaesthesia. After surgery as the sensory block was wearing off, pain was monitored every 15 minutes. When the child was first assessed to have any wound pain, a

blood sample was collected; thereafter pain medication was immediately continued. The study protocol was considered ethical, because the patients were provided with effective pain relief with intravenous paracetamol and ketoprofen, and opioid for rescue analgesia, as soon as they showed the first symptoms of wound pain.

### 6.2 THE TIME COURSE OF DRUG CONCENTRATIONS IN THE CSF

The CNS permeation of NSAIDs and paracetamol has not been adequately described previously. Most previous studies evaluate permeation after intramuscular or oral dosing (Table 9). There are only two adult studies after intravenous paracetamol (Bannwarth et al. 1992, Moreau et al. 1993) and two paediatric studies after intravenous ketoprofen (Kokki et al. 2002, Mannila et al. 2006). Therefore, this is the most extensive study on CNS bioavailability after intravenous NSAIDs. This study shows that most NSAIDs reached the highest concentrations in the CSF at 60 minutes, after which the concentrations began to fall. Indomethacin concentrations in the CSF were variable, and rose faster than those of other NSAIDs. Paracetamol concentrations in the CSF peaked at 1-2 hours. From a clinical perspective, the CNS permeation of NSAIDs and paracetamol is rapid.

In previous studies, the peak concentrations of NSAIDs and paracetamol in the CSF were observed at 2-3 hours (Table 9) (Bannwarth et al. 1990, 1992, 1995, Moreau et al. 1993, Rice at al. 1993, Anderson et al. 1998, van der Marel et al. 2003a, Kozer at al. 2007), whereas in the present study the peak occurred significantly earlier, most commonly at 1 hour. The reason for this is probably the different administration routes (Gibb and Anderson 2008): in the previous studies the drugs were given by mouth (Bannwarth et al. 1995, Anderson et al. 1998, Kozer at al. 2007), intramuscularly (Bannwarth et al. 1990, Rice at al. 1993) or rectally (van der Marel et al. 2003a,), whereas in the present study intravenous dosing was used. In the previous studies the drug had to be absorbed first, and only then could be distributed in the CSF. Typically the absorption of NSAIDs and paracetamol is fast, the peak concentrations in plasma occurring 1-2 hours after intramuscular or oral dosing (Forrest et al. 1982, Davies and Anderson 1997, Gillis and Brogden 1997, Davies 1998), and this fits well with the peak in CSF at 2-3 hours.

The findings of the present study throw light on the optimal timing of preventive analgesic administration. In the present study, NSAIDs and paracetamol were given as intravenous injections over 5-10 minutes, and the highest drug concentrations in the lumbar CSF were observed 1 hour after administration. Based on the assumption that CSF concentrations are more closely related to analgesia than plasma concentrations, the optimal time to administer preventive intravenous analgesics might be 1 hour before the onset of acute pain. The current common clinical practice is to give enteral drugs approximately 1-2 hours before anticipated intense pain, and parenteral drugs only shortly before or at the onset of pain. It is possible that earlier administration of intravenous analgesics would improve pain control on the early phase of acute pain (Rømsing et al. 1998, Møiniche et al. 2002, Kehlet et al. 2006), because of sufficient CNS concentrations. Previous studies on postoperative pain have observed maximal pain relief at 0.5-1 hour after intravenous NSAIDs and paracetamol (Rice et al. 1995, Mandema and Stanski 1996, Granry et al. 1997, Moller et al. 2005, Murat et al. 2005). However, results on pre-emptive use of NSAIDs are contradictory (Norman et al. 2001, Kokki and Salonen 2002, Ong et al. 2005), and furthermore pre- and perioperative administration of NSAIDs is limited by the risk of increased blood loss, and renal adverse effects in the case of hypovolemia and hypotension.

### 6.3 COX INHIBITION AT CONCENTRATIONS OF NSAIDS OBSERVED IN THE CSF

With the doses used in the present study, NSAID concentrations in the CSF are relevant in pain management. The noxious stimulus from the lower part of the body is conducted by Aδ- and C-fibres via the dorsal root ganglia to the dorsal horn of the spinal cord. The first synapse is in the outer part of the dorsal horn, in the substantia gelatinosa, and from there the axons continue with the spinothalamic tract up the contralateral ventral surface of the spinal cord, through the medulla, pons and the midbrain, to the thalamus. Therefore, the synapses and neurons are in close contact with the CSF. Moreover, COX is expressed in the spinal cord constitutively, and also in response to a painful stimulus (Svensson and Yaksh 2002, Zhu et al. 2003).

The issue of sufficient drug concentrations in the CNS is complicated. In the present study, CSF pharmacokinetics, instead of pharmacodynamics using an effect measure (e.g. pain relief), was studied, and therefore no distinct conclusions on this subject can be made. However, some comparisons between the results and those of previous studies are presented here. The results ($IC_{50}$ of COX-1 and COX-2 inhibition) from three previous studies (Mitchell et al. 1993, Warner et al. 1999, Kato et al. 2001) are summarized in Table 2. However, the results are obtained from different study settings, which have their advantages and drawbacks (Pairet and van Ryn 1998), and the results vary a lot.

Indomethacin $IC_{50}$ of COX-1 is 3.2 μg/l (Kato et al. 2001), which is in the range of the observed CSF indomethacin concentrations (range 0.2 – 5.0 μg/l), so it is possible that the observed concentrations yield analgesic actions in the CNS. Ibuprofen $IC_{50}$ for COX-1, according to Mitchell et al. (1993), is twice the observed CSF concentrations in the present study. However, in a study by Cryer and Feldman (1998), $IC_{50}$ for ibuprofen in human gastric mucosa was 140 μg/l, which is in the range of observed ibuprofen concentrations in the CSF. This indicates that the observed concentrations in the present study might be high enough for COX inhibition as well. For ketorolac, the median of observed concentrations in the CSF was ten times higher than its $IC_{50}$ for COX-1 (Warner et al. 1999). In previous studies the $IC_{50}$ for COX-2 of diclofenac was 7.7 μg/l (Kato et al. 2001) and 0.29 μg/l (Kawai et al. 1998), indicating that the observed CSF concentrations, 0.1 – 4.7 μg/l, might be high enough for COX inhibition as well.

### 6.4 PROTEIN BINDING AND DRUG CONCENTRATIONS IN THE CSF

Protein binding seems to be a major factor affecting NSAID CNS permeation (Parepally 2005). It is believed that the BCSFB allows non-protein-bound (free) drug in plasma to reach equilibrium with free drug in the CSF (Figure 20). In the present study, some calculations were performed to approximate NSAID protein binding in the CSF. It seems that the models are applicable to the situation, because the predicted percent bound in the CSF fits fairly well with the findings ofprevious study (Müller et al. 1991) and also with the ratio of total concentration in CSF to unbound concentration in plasma in the present

study. However, applying the equations (Honoré and Brodersen 1984, Borgå and Borgå 1997, Deschamps-Labat et al. 1997, Yamasaki et al. 2000) may have some drawbacks. The models were built to describe NSAIDs binding to albumin in plasma. Here the calculations were extrapolated to the situation in the CSF, with albumin concentration less than 0.5% of that in plasma. Therefore, the results are rough extrapolations, and should be interpreted with caution, and a more detailed model with predictions for individual patients was not built. Furthermore, it is possible that protein binding in the CSF is more extensive in young children, because the concentration of proteins in the CSF is higher in infants than in older children and adults (Biou et al. 2000, Wong et al. 2000).

*Indomethacin*

Indomethacin concentrations in the CSF were three times higher than free concentration in the plasma (median 1.4 and 0.5 μg/l, respectively). Indomethacin binding to proteins in the CSF may explain this phenomenon. Two studies (Honoré and Brodersen 1984, Müller et al. 1991) on the protein binding of indomethacin in the CSF suggest 40% binding. However, this percentage of binding should lead to a CSF/free plasma ratio of 1.7, whereas the median was 3.1 in the present study (range 0.4-15). However, imprecision of the analytical method in the range of free plasma indomethacin concentrations is a possible explanation to higher CSF/free plasma ratios than expected.

*Ibuprofen*

Ibuprofen concentrations in the CSF were two – three times higher than its free plasma concentrations in samples collected after 20-30 minutes (median 182 and 72 μg/l, respectively). The binding equations (Honoré and Brodersen 1984, Deschamps-Labat et al. 1997, Yamasaki et al. 2000) suggest 15-49% binding in the CSF. Fifty percent binding would result in a CSF to free plasma ratio of 2, which is comparable to the observed median of 2.3 in the present study.

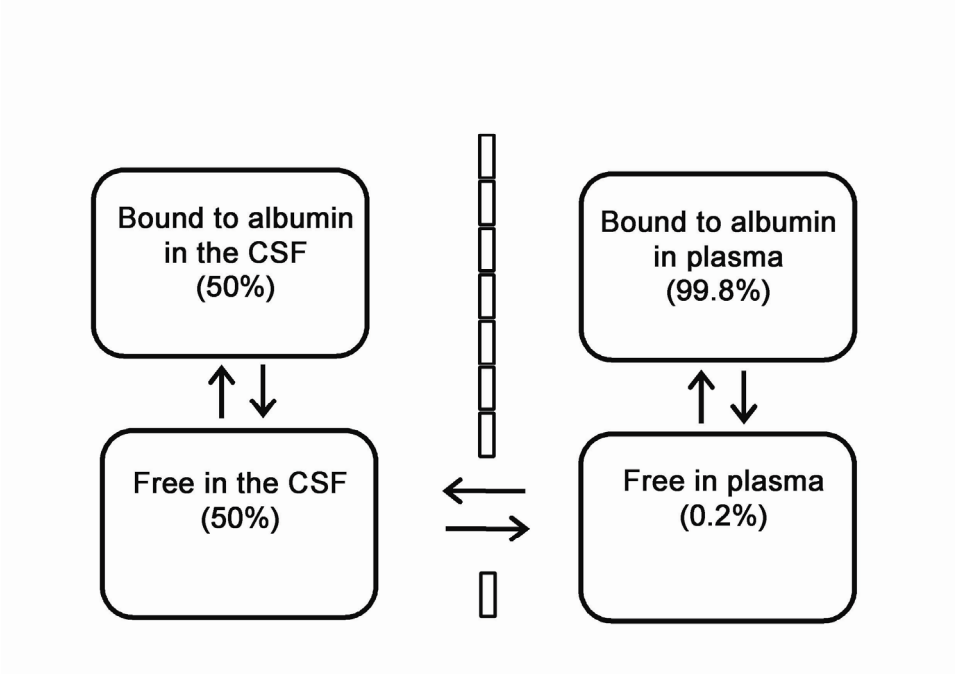


Figure 20. Free ibuprofen in plasma reaches equilibrium with free ibuprofen in the CSF

*Ketorolac*

In contrast to indomethacin, ibuprofen and diclofenac, ketorolac concentrations in the CSF remained below free plasma concentrations at all times (median of the ratio 0.08). This result is rather surprising since ketorolac is also highly protein-bound (99.66%) in plasma, and binding presumably also occurs in the CSF. This finding cannot be compared with results in a previous study, since unbound ketorolac concentrations were not measured in plasma by Rice and coworkers (1993). Moreover, to the best of my knowledge there are no reports on ketorolac transport by efflux or influx proteins that might facilitate ketorolac clearance from the CNS. Therefore, it remains unclear why ketorolac CNS bioavailability is less than that of other NSAIDs.

*Diclofenac*

Diclofenac concentrations in the CSF were two times higher than its free plasma concentrations in samples collected later than 20 minutes after drug administration (median 1.2 and 0.8 μg/l, respectively). The binding models give 56-89% (Honoré and Brodersen 1984, Borgå and Borgå 1997, Yamasaki et al. 2000) protein binding in the CSF. Binding of 65% would be consistent with a CSF to free plasma ratio of 2.9, which is close to the observed median of 2.2 in the present study.

*Paracetamol*

In study V, non-protein-bound paracetamol concentrations in plasma were not measured, because paracetamol is less than 20% bound to erythrocytes and plasma proteins at therapeutic concentrations (Milligan et al. 1994). In the present study, paracetamol concentrations in the CSF reached the level of plasma concentrations at 1 hour and remained at the same level as plasma concentrations thereafter. Clearly distinct CNS bioavailability is seen with paracetamol, when compared with NSAIDs. The difference in permeation is probably due to the low binding of paracetamol in blood (Parepally 2005).

In summary, the results of indomethacin, ibuprofen, diclofenac and paracetamol support the theory of the equilibrium of free concentrations in plasma and the CSF. The calculations on protein binding suggest moderate protein binding in the CSF for indomethacin (40%), ibuprofen (50%) and diclofenac (65%). There are no studies available to base calculations on ketorolac and paracetamol protein binding in the CSF.

The results on ketorolac conflict with the theory of the equilibrium of free concentrations in plasma and the CSF; in this study, ketorolac concentrations in the CSF were 10 times lower than its free plasma concentrations. This finding may explain the high incidence of systemic adverse effects of ketorolac. When the drug is assumed to inhibit COX in the CNS, it clearly inhibits COX in the kidneys and in the gastric wall, as free concentrations in plasma are ten-fold higher.

## 6.5 PHYSICOCHEMICAL CHARACTERISTICS OF NSAIDS AND THEIR CONCENTRATIONS IN THE CSF

The physicochemical characteristics have a major effect on drug permeation through biological barriers (Davson and Segal 1996). In general, NSAIDs are considered to permeate through the barriers easily. The present study suggests that ketorolac permeates the CSF less readily than the other studied NSAIDs. With the lowest pKa dissociation constant, ketorolac is the most ionized drug in plasma pH (Table 8). This could be one explanation for the lower permeation, because it is believed that only the unionized drug in plasma can diffuse through biological barriers.

Lipophilicity is another factor which may account for the lower CSF concentrations of ketorolac. In animals, drugs with logP values between 2.6 and 4.3 entered the CSF easily (Péhourcq et al. 2004). The logP values of indomethacin, ibuprofen and diclofenac are in the upper range (4.3, 3.5, and 4.4, respectively). In contrast, the logP value of ketorolac is in the lower range (2.7). Therefore, it is possible that ketorolac diffuses in the CSF to a smaller degree because of its lower lipophilicity.

## 6.6 AGE- AND SIZE-RELATED DIFFERENCES IN DRUG CONCENTRATIONS IN THE CSF

The present study indicates that there might be age-related differences in the CSF permeation of NSAIDs in children. In the present study, younger children had higher drug concentrations in the CSF after indomethacin and diclofenac than did older children. Age, height and weight were not correlated with ibuprofen and paracetamol CSF concentrations.

*Indomethacin*

There was a relationship between CSF indomethacin concentrations and the age, height, weight and body surface area of the children. Previously CSF indomethacin concentrations have been studied in 52 adults (Bannwarth et al. 1990). The highest concentrations in the CSF in children and adults are comparable, when differences in the body weight-based doses are taken into

account. The lag time to reach the peak CSF concentration in adults probably reflects the lag in absorption after intramuscular administration and also slower CSF permeation, as teq usually scales with weight$^{0.25}$ (Anderson and Meakin, 2002). Indomethacin might permeate the CSF faster in smaller children than in heavier children and adults.

*Ibuprofen*

The children's weight and age were not related to ibuprofen concentrations in the CSF. Previously, ibuprofen concentrations in the CSF have been studied in 46 adult patients (Bannwarth et al. 1995). The maximal observed concentrations in the CSF were similar in adults and children after a similar mean weight-adjusted dose of ibuprofen. The peak concentration was reached later in adults, probably because of the lag of absorption after oral dosing and slower permeation.

*Diclofenac*

Diclofenac concentrations in the CSF have been previously measured in only two adult patients (Zecca et al. 1991). In those adults, diclofenac CSF concentrations were similar to those observed in children in the present study. In the present study, smaller children had higher diclofenac concentrations in the CSF than did heavier children. It seems that CSF permeation is faster in smaller children than in heavier children and adults.

*Ketorolac*

Regression analysis to evaluate the effect of age, height, weight and body surface area on the CSF concentrations of ketorolac was unsuccessful. When comparing the results of the present study and the previous study, ketorolac concentrations in the CSF were four times lower in children than in adults (Rice et al. 1993), probably because of the 2.4-times higher im weight-based dose in adults. The higher volume of distribution in children (Forrest et al. 1997, Hamunen et al. 1999) may also account for the difference. Moreover, CSF to total plasma concentration ratio was similar in children and adults, and therefore it seems that there are no major differences in the CSF ketorolac concentrations between children and adults. However, based on the theory of

faster CNS distribution of drugs in smaller children than heavier children (Anderson and Meakin, 2002), it is possible that ketorolac also permeates faster into and out of CSF in smaller children than in heavier children and adults.

*Paracetamol*

Based on previous reports, it seems that paracetamol permeates the CSF more rapidly in younger children with lower body weight than in older children with higher weight. Previously, a shorter CSF equilibration half-time was found in children with head traumas (0.7 h) (Anderson et al. 1998) than in adults (2.1 h) (Bannwarth et al. 1992). However, the children in Anderson's study had disrupted BBB function because of brain injury. In a larger study in children without BBB disruption, it seemed that size rather than age explains the shorter CSF equilibration half-time (van der Marel et al. 2003a), and 70-kg standardized teq (1.9 h) was similar to that calculated from the adult data (2.1 h) (Bannwarth et al. 1992). The results of the present study are at variance with those of the previous studies, since no size- or age-related difference was found in paracetamol concentrations in the CSF in the present study in 32 children aged 3 months to 12 years weighing 7-69 kg. However, in the present study, modelling and teq calculation was not performed, and it is possible that differences in permeation times may not have been detected. In a previous study by Kozer et al. (2007), no age difference was found, because of the variable dose and small age range (1 week – 9 months).

The CSF concentrations after paracetamol administration were higher in children than in adults. After the administration of the prodrug propacetamol in adults, paracetamol concentrations in the CSF were maximally 9 mg/l (Bannwarth et al. 1992), whereas that level was clearly exceeded in the present study (highest 18 mg/l, median 7.2 mg/l) and in the previous studies in children after rectal and oral dosing (van der Marel et al. 2003a, Kozer et al. 2007). However, the ratios of paracetamol concentrations in the CSF and in plasma were similar in adults (Bannwarth et al. 1992) and in children (the present study) (Figure 21). Nevertheless, there is some indication of a difference between adults and children: children may have higher CSF to plasma ratios during 60-120 minutes (Figure 21), in agreement with shorter teq in children (Figure 22). Paracetamol dosing was similar in these studies. In the

present study, intravenous paracetamol was used, and in the adult study (Bannwarth et al 1992), paracetamol was administered as prodrug propacetamol, which is rapidly and completely hydrolyzed to paracetamol in plasma within 20 minutes (Granry et al. 1997). Moreover, in the present study in children the dose was 15 mg/kg and in the previous study in adults 2 g propacetamol corresponding mean paracetamol 13.9 mg/kg. The doses per kilogram were similar, although the per-kilogram model is not ideal (Anderson and Meakin, 2002). These paracetamol doses resulted in different plasma concentrations, which could account for the difference in the level of CSF concentrations. In the adult study, paracetamol concentrations in plasma were markedly lower (mean 8.2 mg/l at 1 hour) than in the present study (mean 15.5 mg/l at 1 hour). Furthermore, similar CSF to plasma paracetamol ratios support this theory. However, since pharmacokinetic modeling and teq calculation was not performed in the present study, differences in teq between adults (Bannwarth et al 1992) and children (present study) are not confirmed. It seems that paracetamol permeates the CSF faster in small children than in bigger children and adults, but the clinical significance and implications of this remains unclear.

In summary, this study indicates that infants and small children may have higher CSF concentrations of NSAIDs than bigger children. This difference can be explained with an allometric ¼ power model, which suggests that CSF equilibrium half-life scales with weight$^{0.25}$ (Anderson and Meakin, 2002). It is possible that immaturity of BCSFB function (Saunders et al. 1999), increased cerebral blood flow (Zwienenberg and Muizelaar, 1999), lower albumin content of plasma (Gomez et al. 1984), higher albumin content of the CSF (Biou et al. 2000, Wong et al. 2000) or other differences in drug kinetics have effects in the observed higher NSAID concentrations in the CSF in infants. However, this study was not designed to study the effect of age on drug concentrations in the CSF, so the sampling times were unevenly distributed in different age groups and the used statistical methods have some limitations. Therefore, the results of the present study should be considered preliminary. Moreover, in the present study paracetamol CSF kinetics was not correlated with age. Based on previous studies, size-dependent difference in paracetamol CSF kinetics are likely, but these size-related differences in CNS-kinetics of NSAIDs and paracetamol have not affected clinical practice.

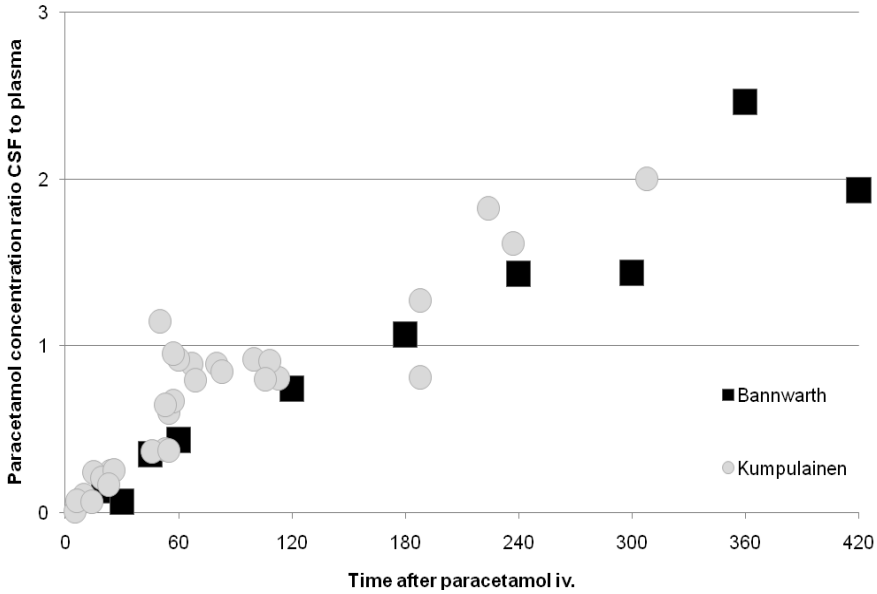


Figure 21. Paracetamol concentration ratios CSF to plasma in adults (Bannwarth et al. 1992) and in children in the present study (Kumpulainen et al. 2007, V). Grey circles represent concentration ratios of individual patients in the present study, and black squares represent ratios of mean CSF and mean plasma concentrations in Bannwarth et al. (1992).

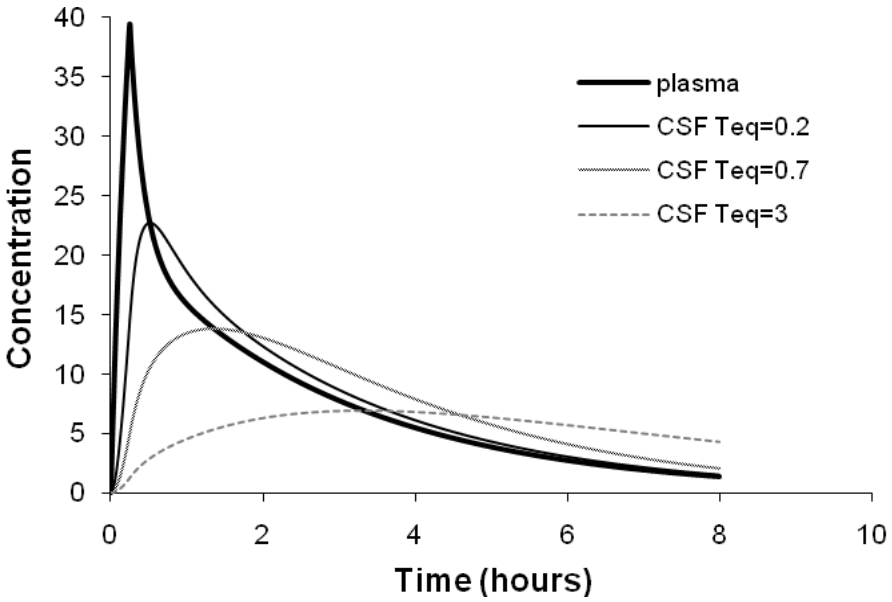


Figure 22. Simulation of paracetamol concentrations in the CSF after intravenous administration, with three different equilibration half-times (teq), measured in hours. The shorter teq results in an earlier and higher peak concentration in the CSF for the same dose.

The simulation is for a child weighing 20 kg, after a 15-min intravenous infusion of paracetamol 15 mg/kg. The model was a two-compartment model (including central $V_1$ and peripheral $V_2$ compartments), with an additional CSF $V_3$ compartment. The differential equations were:

$$\frac{d(A1)}{dt} = ratein + Q2 \times C2 - (CL + Q2) \times C1$$

$$\frac{d(A2)}{dt} = Q2 \times (C1 - C2)$$

$$\frac{d(A3)}{dt} = Keq \times (C1 - C3)$$

where A1 is the amount of paracetamol in the central compartment, A2 is the amount in the peripheral compartment, A3 is the amount in the CSF…

(Continues on the next page)

(Figure 22)

… Q2 is the intercompartmental clearance between the central and peripheral compartments, CL is the clearance from the central compartment, C1 is the concentration of paracetamol in the central compartment, C2 is the concentration in the peripheral compartment, C3 is the concentration in the CSF compartment, Keq is the equilibration rate constant between the central and the CSF compartments. Partition coefficient was assumed 1. For parameter values see Anderson et al. (2005).

### 6.7 SEX DIFFERENCE IN DRUG CONCENTRATION IN THE CSF

Previous reports indicate that there may be gender or sex differences in response to analgesics (Ciccone and Holdcroft 1999, Greenspan et al. 2007). An association of sex and drug concentrations in the CNS has not been reported earlier. In this study, girls had higher paracetamol concentrations in the CSF than boys. However, the study was not powered and designed to study sex difference of drug concentrations in the CSF: the sample size was small and sampling times were unevenly distributed between girls and boys. In previous studies in children (Anderson et al. 1998, van der Marel et al. 2003a, Kozer et al. 2007), no sex difference in paracetamol concentrations in the CSF was found. Therefore, it seems that there are no true differences in CNS pharmacokinetics between boys and girls.

Some previous studies indicate that minor differences in paracetamol pharmacokinetics in women may occur in connection with the menstruation cycle (Wójcicki et al. 1979, Gugilla et al. 2002). Furthermore, female infants up to three months of age have been shown to have higher estradiol levels than older children (Chada et al. 2003) or males (Schmidt et al. 2002), which might also suggest a sex difference in paracetamol kinetics. However, most previous studies on paracetamol pharmacokinetics (Forrest et al. 1982, Anderson et al. 1998, 2004, 2005, van der Marel et al. 2003b, Jacqz-Aigrain and Anderson 2006, Duggan and Scott 2009) suggest that no sex difference in pharmacokinetics occur.

### 6.8 PROTEIN BINDING IN PLASMA

In the present study, NSAIDs were highly bound to proteins in plasma. Indomethacin was 99.96%, ibuprofen 99.80%, ketorolac 99.66% and diclofenac 99.90% bound to proteins. The results are similar to previous findings, where protein binding was also high: indomethacin >99.7% (Bannwarth et al. 1990), ibuprofen >99% (Davies 1998), ketorolac >99% (Gillis and Brogden 1997) and diclofenac >99.7% (Davies and Anderson 1997). As discussed previously, protein binding in plasma is a major factor affecting NSAID CNS permeation. In the present study, the children were healthy, undergoing elective surgery and presumably had normal plasma albumin concentrations. However, in children suffering from malnutrition, and even in those with an acute illness, plasma albumin concentrations decrease (Potter and Luxton 1999). This leads to decreased NSAID binding and increased unbound drug concentration, and consequently increased diffusion to the CNS.

### 6.9 ANALGESIC CONCENTRATIONS IN PLASMA

Pain occurrence after inguinal surgery was closely monitored in three (II) and eight (IV) children to get a plasma sample at onset of pain. The children had spinal anaesthesia with standard weight-based doses of levobupivacaine, light sedation for the operation and preoperatively one dose of ibuprofen or diclofenac. However, these results should be considered preliminary. No conclusions based on the results can be drawn, since the sample size was too small and the timelines of different events affecting the time course of pain (drug administration, spinal anaesthesia, sedation, the beginning and the end of operation) were variable. Nevertheless, it seems that pain occurrence after spinal anaesthesia varies, and therefore pain medication should be administered preventively and based on individual needs.

### 6.10 THE ADVERSE EFFECTS

A total of 41 adverse effects were reported in 40 out of 160 children, but no serious or unexpected adverse effects were noted. Because the study design

was open, the observations may have bias. Moreover, no follow-up for adverse effects after discharge was arranged.

CNS adverse effects (agitation and anxiety) occurred in 19 children (12%) in the PACU. Emergence agitation is a common adverse effect after inhalation anaesthesia with sevoflurane (Goa et al. 1999, Kuratani and Oi 2008), and restlessness has also been reported after spinal anaesthesia, with an incidence of 7% (Kokki and Hendolin 1996). NSAIDs, especially indomethacin, may cause CNS adverse effects, headache, dizziness, anxiety, agitation and cognitive dysfunction (Tharumaratnam et al. 2000, Clunie et al. 2003). In this study, 5/31 children who had received indomethacin and 7/30 children who had received ketorolac experienced CNS adverse effects. Because anaesthesia and sedation was standardized, the CNS adverse effects may be connected to the study drugs.

Gastrointestinal adverse events (vomiting 5%, nausea 4%, abdominal pain 1%) occurred at similar incidences as reported in a previous study in children with spinal anaesthesia (Kokki and Hendolin 1996), and may be related to the study drugs. Urticaria occurred in one child after the child had received intravenous ketorolac and a concomitant buccal midazolam-ketamine premedication, and may be related to ketorolac. Shivering was recorded in four children (2.5%), at a similar incidence as reported by Kokki and Hendolin (1996). Shivering is common after spinal anaesthesia in children (Kokki and Hendolin 1996, Crowley and Buggy 2008), and was probably not related to the study medications.

### 6.11 FUTURE PERSPECTIVES

In the present study, concentrations of NSAIDs and paracetamol were studied after a single intravenous dose. In the future it would be interesting to build a population pharmacokinetic model using the data of the present study. With the model it would be possible to evaluate the effect of size on CSF equilibrium half-life and to simulate CNS concentrations after repeated doses and continuous infusion. Moreover, a further study of the CNS kinetics of ketoprofen is warranted, since Mannila et al. (2006) sampled the CSF only up to 67 minutes, with increasing ketoprofen concentrations. It would be useful to

find out whether the peak in the CSF occurs at 1 hour or later after intravenous ketoprofen, and to gain more knowledge of the elimination of ketoprofen from the CSF. Furthermore, the concentrations of non-opioid analgesics in the tissues of the CNS should be investigated, using microdialysis methods, for example. However, the value of future clinical trials on CNS kinetics is controversial; it is hard to see how such studies would change clinical practice or fundamental concepts of the use of the drugs. On the other hand, pharmacodynamic trials studying the relationship of CNS kinetics and analgesia would be difficult to conduct, but could improve clinical practice. Moreover, when paediatric CNS bioavailability is considered, it seems that CSF equilibrium half-life (teq) is related to size, but other differences are small, and therefore the justification of paediatric clinical trials on CNS kinetics is questionable.

The findings of the present study do, however, support the concept of preemptive analgesia. There may be delays in the onset of analgesia after oral administration, partly due, to the time needed for absorption from the gut into the plasma. This thesis also documents CSF delays after intravenous administration due to the delay between plasma and CSF. CSF is mooted to be the site of action of both NSAIDs and paracetamol. These drugs should be given intravenously about 30 min before the onset of pain (e.g., 30 min before arriving in the recovery room after a surgical procedure). The delay between administration and effect is greater after oral administration, and this affects the timing of oral doses that are best given before the surgical procedure.

# 7 Summary and conclusions

NSAIDs and paracetamol have an analgesic effect in the CNS mediated through prostaglandin H2 synthetase (PGHS) enzyme inhibition. However, the CSF distribution of these analgesics in healthy children has not been previously studied. NSAIDs are highly protein bound to albumin (>99%). Concentrations of indomethacin, ibuprofen, ketorolac, diclofenac and paracetamol in the CSF were studied in 160 children. CSF was sampled during spinal anaesthesia 5 min–22 h after intravenous analgesic injection. The following conclusions can be made:

- All the investigated drugs exhibited permeability of the BBB and were detectable within the CSF during the study period, consistent with their centrally mediated mechanism of action.

- The highest concentrations of ibuprofen, ketorolac, diclofenac and paracetamol in the CSF occurred within one hour after intravenous administration. CSF concentrations of indomethacin were higher than unbound plasma concentrations over the study period, suggesting rapid distribution to the CSF (peak <30 min).

- Total CSF/unbound plasma concentrations were greater than unity for indomethacin, ibuprofen and diclofenac, consistent with the theory of equilibrium between unbound concentration in plasma and the CSF. CSF proteins, albeit less than plasma, contribute to binding in the CSF (approx. 15-69%) and cause higher total CSF drug concentration.

- CSF/unbound plasma ketorolac concentration ratios were below one; the reason for this may be ionization, less lipophilicity or active transport.

- Paracetamol concentrations in the CSF are similar to those in plasma after one hour, consistent with its low degree of binding in blood (<50%).

- Age-related differences in NSAID or paracetamol CNS bioavailability were not detectable with the current study protocol.

Concentrations of NSAIDs in CSF are likely to have a closer temporal relationship with analgesia than those in plasma. The time of analgesic delivery should be tailored so that peak CSF concentrations precede the onset of maximum pain, e.g. emergence from anaesthesia where a regional block is not used. Further clinical studies are required to confirm this hypothesis.

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**Elina Kumpulainen**

# *Central Nervous System Permeation of Non-Steroidal Anti-Inflammatory Drugs and Paracetamol in Children*

Non-steroidal anti-inflammatory drugs and paracetamol are commonly used analgesics in acute pain management. In the present study the permeation of these drugs into the central nervous system was evaluated in 160 healthy children. Diclofenac, ibuprofen, indomethacin and ketorolac permeated the cerebrospinal fluid readily and reached the highest concentrations one hour after intravenous dosing. However, the concentrations were 100-fold lower when compared to that in plasma. Paracetamol performed differently: the cerebrospinal fluid concentrations reached the level of plasma concentrations at one hour. These results suggest that the optimal timing for intravenous administration of non-opioid analgesics is an hour before the onset of acute pain.

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